THE YEASTS

A TAXONOMIC STUDY

Fourth Revised and
Enlarged Edition

Editors

C.P. Kurtzman
J.W. Fell

ELSEVIER

The Yeasts, a taxonomic study

This book is dedicated to the memory of

Anna Kocková-Kratochvílová

Jacomina Lodder

Takeshi Tsuchiya

Nicolau J. van Uden

Lynferd J. Wickerham

The Yeasts, A Taxonomic Study

Fourth edition

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Preface

The importance of yeasts is underscored by our often daily consumption of bread as well as fermented beverages. Recent advances in biotechnology have increased reliance on yeasts for pharmaceuticals and for bulk biochemicals such as citric acid. Furthermore, clinically important yeasts are commonplace, especially with increasing numbers of immune-suppressed patients, and biologists are continuing to discover the importance of yeasts in the ecosystem. All of these areas of science and technology have a common need, the rapid and accurate identification of yeasts. The goal of this book is to provide that information.

This book, the fourth edition of *The Yeasts, A Taxonomic Study*, represents a continuation of the monographic series begun by J. Lodder and N.J.W. Kreger-van Rij (1st edn., 1952), J. Lodder (2nd edn., 1970) and N.J.W. Kreger-van Rij (3rd edn., 1984). As with each successive edition, the number of taxa treated has increased. In the third edition (1984), 60 genera and 500 species were described, whereas in the fourth edition, there are 100 genera representing over 700 species. Numbers alone do not fully reflect the increased coverage of the present treatment. Besides newly described species, the current edition includes additional genera of yeastlike taxa, some of which, such as *Ascoidea* and *Dipodascus,* are now recognized as members of the ascomycetous yeast clade, whereas others, such as *Trichosporonoides* and *Prototheca,* are not considered to be yeasts but are included because they are morphologically similar to yeasts and sometimes confused with them. Similarly, we broadened the discussion of the basidiomycetes to acquaint the reader with some of the dimorphic species whose yeast phases are often found on isolation plates. Molecular studies have shown many of these dimorphic species have close phylogenetic relationships with the commonly recognized basidiomycetous yeasts.

Not surprisingly, molecular sequence analyses have contributed prominently to the classification system used in the fourth edition. However, because sequence data are not yet available for all known species, a phylogenetically-based classification system is not fully developed. As a consequence, the placement of many species in genera and families is uncertain. Despite these limitations, the system of classification presented in the current edition is more predictive of natural relationships than was possible in earlier treatments.

The current edition features an expanded list of assimilation tests over that of earlier editions. Besides providing increased diagnostic resolution of taxa, the additional physiological data will aid biotechnologists searching for species that are capable of unique biochemical transformations.

This edition includes chapters on the importance of yeasts and on current methods used for their classification. We hope readers will find these chapters useful, and that they will provide a start for more extensive study with a fascinating group of fungi that we know as the yeasts.

Cletus P. Kurtzman

Jack W. Fell

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Use of this book

1. Introductory chapters

The first four sections of this book provide an overall introduction to the yeasts. Section I discusses the current definition of yeasts, their classification, and the rules for their nomenclature. Section II focuses on the importance of yeasts as human, animal, and plant pathogens, their role in biotechnology, and their ecological importance. Section III provides chapters on the ultrastructural, biochemical and molecular biological properties that are currently being used to recognize yeasts and to develop a framework for their classification. Methods for isolation and identification of yeasts are given in Section IV along with protocols for various biochemical analyses that are used for characterization of species.

2. Chapters on the genera and species

The arrangement of taxa in the fourth edition is somewhat different from that of previous editions. Because it is possible to determine whether isolates are ascomycetes or basidiomycetes from the diazonium blue B (DBB) test and from ultrastructure of cell walls, anamorphs can now be placed in their appropriate taxonomic class. Consequently, the taxonomic descriptions start with ascomycetous yeasts that have known sexual states, followed by the anamorphic ascomycetous genera. In turn, the teleomorphic basidiomycetes are described followed by their anamorphs. In previous editions, anamorphs from both classes were placed in the same section. The introductory chapters for both the ascomycetes and the basidiomycetes include brief diagnostic descriptions of each genus followed by a key to genera. A key to the anamorphic genera of yeastlike archi- and euascomycetes also has been provided.

3. Descriptions of species

Each species description begins with a designation of the anamorph or teleomorph, where known, followed by a listing of synonyms. The characterization proceeds to morphological and physiological descriptions. Noteworthy information for the species is given in the section entitled "Comments". Each genus description concludes with the section entitled "Comments on the genus", where the history of the genus is given along with a discussion of its classification. Many of the comments sections also provide information of interest to biotechnologists, clinicians and ecologists.

The following symbols are used for the fermentation and assimilation reactions given with species descriptions:

Symbol	Meaning
+	positive
l	latent (rapidly developing a positive reaction after a lag)
+/l	positive or latent
s	positive but slow
w	weak
ws	weak and slow
+/w	positive or weak
w/–	weak or negative
lw	latent but weak (rapidly developing a weak reaction after a lag)
–/l	negative or latent
v	variable
–	negative
n	no data

Representative species have been illustrated for each genus. The illustrations, which may be either drawings or photographs, included a scale marker bar and growth conditions.

A summary table of fermentation and assimilation reactions and certain key biochemical characteristics has been compiled. For easy access, taxa are listed alphabetically, first by genus and then by species. The following abbreviated symbols are used in this table:

+	+, s, l, +/l	×	+/w
–	–	v	v, w/–, –/l
w	w, ws, lw	n	no data or not applicable

4. Keys to taxa

Keys to genera are given in the introductory chapters to the ascomycetes and the basidiomycetes, and keys to the species assigned to a particular genus are given in the chapter discussing that genus. A key has also been constructed that includes all species of ascomycetes and basidiomycetes for which there are available data. In addition to the preceding, the following specialized keys have been developed: key to selected human- and animal-associated yeasts (see chapter 2: yeasts pathogenic for humans); key to anamorphic genera of yeastlike archi- and euascomycetes (chapter 16); key to yeasts forming ballistospores (chapter 76).

5. Yeastlike taxa

Some of the yeastlike taxa likely to be mistaken as yeasts are briefly discussed in this book. For example, species of the genus *Aureobasidium* are commonly isolated dimorphic euascomycetes that often appear white to light pink in color and yeastlike on isolation plates. Similarly, the achlorophyllic algal genus *Prototheca* is often misidentified as a yeast, and for this reason a chapter on the genus is included.

6. Indexes to taxa

The fourth edition contains two indexes to taxa. The first of these is a standard index that lists genera followed by assigned species and their synonyms. Validly accepted combinations are given in bold type. The second index alphabetically lists all species and variety names followed by all genera to which the species and varieties were ever assigned. Validly accepted genera are given in bold type.

7. Glossary

A glossary has been provided that includes morphological, genetic, and molecular biological terms.

8. References

The references for all chapters have been consolidated into a single list. By using a consolidated list, sufficient space was saved that titles could be included, whereas this would not have been possible if each chapter had a reference list.

Part I

Classification of yeasts

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Chapter 1

Definition, Classification and Nomenclature of the Yeasts

C.P. Kurtzman and J.W. Fell

Contents

1. Definition and classification of the yeasts

The English word yeast and its equivalents in many other languages are based on words meaning foam and to rise, direct references to the fermentation processes giving beer and bread. For this reason yeasts are often thought of as fermentative ascomycetous fungi similar to *Saccharomyces cerevisiae*. In fact, it is not uncommon in some areas of molecular biology to treat the words "yeast" and "*Saccharomyces*" as synonyms. The discovery that some taxa are basidiomycetes has broadened our perception of the nature of yeasts. As a consequence, we have come to view yeasts as fungi with vegetative states that predominantly reproduce by budding or fission, resulting in growth that is comprised mainly of single cells.

An imprecise distinction has been made between yeasts and those dimorphic filamentous fungi that oftentimes produce abundant yeastlike growth. Yeasts can be defined as those fungi whose vegetative growth predominantly results from budding or fission and which do not form their sexual states within or upon a fruiting body. For ascomycetous yeasts, this distinction has been substantiated by molecular comparisons, which demonstrate the budding and fission yeasts to be phylogenetically distinct from one another and from the euascomycetes (see chapter 15). One exception is the genus *Eremascus*, which has unenclosed asci, but budding cells are not formed. A similar distinction can be made for basidiomycetous yeasts, which appear phylogenetically separate from the mushrooms and other taxa that form complex fruiting bodies (see chapter 74). In summary, yeasts, whether ascomycetes or basidiomycetes, are generally characterized by budding or fission as the primary means of vegetative reproduction and have sexual states that are not enclosed in fruiting bodies.

2. Taxonomy

Rules for taxonomy of the yeasts and other fungi fall under the authority of the International Code of Botanical Nomenclature. The most recent version of this Code (Greuter et al. 1994) was adopted at the Fifteenth International Botanical Congress, Yokohama, Japan, in 1993, and has been published by Koeltz Scientific Books, D-61453 Königstein, Germany. The following is a brief discussion of the Botanical Code as it applies to yeasts.

2.1. Description of new taxa

2.1.1. Species: Publication of new species must include a description of essential characters as well as a diagnosis that distinguishes the taxon from previously described species. Since January 1, 1935, the description and/or diagnosis must be given in Latin. Failure to comply with this requirement results in an invalidly described species termed a "nomen invalidum" (nom. inval.). A nomen invalidum also results if publication is not in a recognized scientific journal, e.g., as in a patent or a trade magazine. If the new species is designated without a description or a diagnosis, it is invalid and termed a "nomen nudum" (nom. nud.). Names of taxa must be given in Latin or modified in such a way that they follow the rules of Latin derivation including appropriate gender designations. If a name has been incorrectly crafted, it may be treated as an "orthographic error" and corrected. An example is *Pichia membranifaciens* for which the 1888 spelling "*membranaefaciens*" has been corrected in this book. The authority name does not change due to the spelling correction. Other requirements for valid publication include deposition of type material in a publicly accessible herbarium. This material must be an original specimen of the organism, and it is to be dead and dried. The 1994 Code now allows lyophilized specimens to be valid type material (holotype) and that living cultures derived from the lyophilized material are considered *ex typo*, i.e., from the type. It seems that once the original material is exhausted, there is no longer type material available. A possible solution to this problem would be to lyophilize new material and designate it as a neotype, a convention permitted when the original type material is lost or destroyed and the species can be otherwise recognized. This discussion leads to the recognition that a majority of presently accepted yeast species are technically invalid because legitimate type material has not been preserved. A portion of presently lyophilized stocks in culture collections should be held from distribution and designated as neotypes. Consequently, the designation of "Type strain" given for each cultivatable species described in this book

Table 1
Major yeast culture collections

Country	Collections
USA	Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604
	American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville, MD 20852
	Culture Collection, Department of Food Science and Technology (FST-UCD), University of California, Davis, CA 95616
	Yeast Genetics Stock Center (YGSC), Department of Biophysics and Medical Physics, University of California, Berkeley, CA 94720
Italy	Industrial Yeasts Collection (DBVPG), Dipartimento di Biologia Vegetale, Sez. Microbiologia Applicata, Università di Perugia, Borgo XX Giugno 74, 06121 Perugia
Japan	Institute for Fermentation (IFO), 17-85 Juso-honmachi 2-chome, Yodogawa-ku, Osaka 532
	Japan Collection of Microorganisms (JCM), RIKEN, Hirosawa, Wako-shi, Saitama 351-01
Netherlands	Centraalbureau voor Schimmelcultures (CBS), Yeast Division, Julianalaan 67, 2628 BC Delft
People's Republic of China	Center for Collection of General Microbiological Cultures (CCGMC), Institute of Microbiology, Academia Sinica, P.O. Box 2714, Beijing
Portugal	Portuguese Yeast Culture Collection (PYCC), Biotechnology Unit, New University of Lisbon, Quinta da Torre, 282J Monte de Caparica
Germany	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Braunschweig
Russia	Department of Type Cultures of Microorganisms, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushino, Moscow region, 142292
Slovakia	Slovak Collection of Yeasts (CCY), Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 84238 Bratislava
	Research Institute for Viticulture and Enology (RIVE), Matuskova 25, 83311 Bratislava
United Kingdom	National Collection of Yeast Cultures (NCYC), AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA

can, at best, represent a neotype. The 1994 Code does recognize the need for living cultures in the practice of modern taxonomy and states in Recommendation 8B.1: "Whenever practicable a living culture should be prepared from the holotype material of the name of a newly described taxon of fungi or algae and deposited in at least two institutional culture or genetic resource collections. (Such action does not obviate the requirement for a holotype specimen under Art 8.2)." The 1994 Code further states in Recommendation 8B.2: "In cases where the nomenclatural type is a culture permanently preserved in a metabolically inactive state (see Art. 8 Ex. 1), any living isolates obtained from that should be referred to as "ex-type" (*ex typo*), "ex-holotype" (*ex holotypo*), "ex-isotype" (*ex isotypo*), etc., in order to make it clear they are derived from the type but are not themselves the nomenclatural type." From these recommendations, it is clear that the Code strongly encourages scientific cooperation and communication through active sharing of published taxonomic specimens. A listing of commonly used yeast culture collections is given in Table 1.

2.1.2. Genera, families, orders: The rules for describing new genera, families and orders are similar to those for describing new species. These taxa must be based on a validly described species and provided with a Latin description and diagnosis. The rules of priority are briefly described below, but one exception is that orders are exempt from priority usage.

2.2. Basionyms, synonyms, priority of usage

Because of the inexact art of species characterization, as well as the occasional situation in which two independent investigators describe the same new species, the Botanical Code has provided a set of rules to reconcile resulting problems. The following example should prove helpful to the reader, but the Code needs to be consulted for full details.

1 *Saccharomycopsis fibuligera* (Lindner) Klöcker (1924)

Synonyms

2 *Endomyces fibuligera* Lindner (1907)
3 *Endomycopsis fibuligera* (Lindner) Dekker (Stelling-Dekker 1931)
4 *Pichia fibuligera* (Lindner) Boidin, Pignal, Lehodey, Vey & Abadie (1964)
5 *Endomyces lindneri* Saito (1913)
6 *Saccharomycopsis lindneri* (Saito) Klöcker (1924)
7 *Endomycopsis fibuligera* (Lindner) Dekker var. *lindneri* Dekker (Stelling-Dekker 1931)
8 *Endomyces hordei* Saito (1914)
9 *Saccharomycopsis hordei* (Saito) Klöcker (1924)

10 *Endomycopsis fibuligera* (Lindner) Dekker var. *hordei* (Saito) Dekker (Stelling-Dekker 1931)
11 *Candida lactosa* Dwidjoseputro & Wolf (1970)

In the example, *Saccharomycopsis fibuligera* (1) is the currently accepted name. The species was originally described by Lindner in 1907 as *Endomyces fibuligera* (2) and transferred to *Saccharomycopsis* by Klöcker in 1924. Other authors transferred the species to *Endomycopsis* (3) and to *Pichia* (4), but in all cases, the original author, Lindner, is listed in parentheses. The other species listed (5–11) are misidentified strains of *S. fibuligera*. The genus assignments for some of these "species" were also changed before it was recognized that they were conspecific with *S. fibuligera*. In the context of the Code, *Endomyces fibuligera* (2) is the basionym or basal name for the species. Synonyms 2, 3 and 4 are nomenclatural or obligate synonyms of *S. fibuligera* because they are all based on the same type strain. Synonyms 5–11 are termed taxonomic or facultative synonyms because they are based on different type strains. Species 6 and 7 represent obligate synonyms of *Endomyces lindneri*, hence the listing of synonyms is ordered by priority of publication date of obligate synonyms followed by publication date of facultative synonyms and their own obligate synonyms. The Code requires, with few exceptions, that the first described name has priority of usage. Thus, *Endomyces fibuligera* Lindner (1907) is the basionym, and personal preference for another name to serve as basionym, e.g., *Endomyces lindneri* Saito (1913), is generally not allowed. Arguments for exceptions might include uncertain names and the substitution of economically or medically important names for an older but obscure basionym.

The following citations provide examples of descriptions of new taxa and the conservation of older names, but the most recent edition of the Botanical Code should be consulted as well.

New species and genera

Fell, J.W., A.C. Statzell, I.L. Hunter and H.J. Phaff. 1969. *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. Antonie van Leeuwenhoek 35, 433–462.

New combinations

Kurtzman, C.P. 1995. Relationships among the genera *Ashbya, Eremothecium, Holleya* and *Nematospora* determined from rDNA sequence divergence. J. Ind. Microbiol. 14, 523–530.

Conservation of taxa

Fell, J.W., C.P. Kurtzman and K.J. Kwon-Chung. 1989. Proposal to conserve *Cryptococcus* (fungi). Taxon 38, 151–156.

Because the Botanical Code requires an accounting of names previously used, taxonomy sometimes appears fickle. A more positive view is that lists of synonyms document the clarification of species relationships over time. If we are to accept this latter view, we are also obligated not to make name changes in haste as our science progresses.

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Part II

Importance of yeasts

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Chapter 2

Yeasts pathogenic for humans

D.G. Ahearn

Contents

1. Description

The principal yeasts pathogenic for humans are *Candida albicans* and *Cryptococcus neoformans. Candida albicans* is an asexual, diploid (possibly aneuploid), plemorphic fungus with an ascomycetous-type cell wall. This species is endogenous in the oral-, gastrointestinal-, or urinogenital tracts of humans and other warm blooded animals. *Candida albicans* may be categorized as an obligate commensal because its host-free occurrence is relatively rare. Probably 40 to 60% of the adult population harbors *C. albicans.* Infections are usually of endogenous origin, but patient to patient spread can occur (Ahearn 1988, Jones 1990).

The syndromes produced by *Candida albicans* are highly diversified including vulvovaginitis, dermatitis, cystitis, fever, myositis, hepatic disfunction, and mental confusion. These may occur singly or in combinations dependent upon the superficial, locally invasive, or deep nature of the infection. *Candida albicans* is involved in millions of superficial infections per year (mainly vulvovaginitis) and in over 90% of the systemic or deep yeast infections (estimated in the USA for the 1980's to range from 5000 to 8000 per year). Strains of *C. albicans* vary in their virulence, but as with all yeast infections, the balance between transient or commensal colonization or parasitism is dependent upon the physiological status of the host. Chronic superficial candidiasis is often associated with malnutrition, pregnancy, and diseases with impaired immune systems such as diabetes and malignant neoplasms. Oral candidiasis and localized esophagitis show a particularly high frequency among patients infected with the human immunodeficiency virus (Phelan et al. 1987). Recently, a yeast phenotypically similar to *C. albicans*, but apparently genetically distinct, *C. dubliniensis*, has been associated with AIDS (Sullivan et al. 1995). Candidiema may be indicative of deep or systemic yeast infections involving two or more organs. Such grave infections are frequently iatrogenic resulting from hyperalimentation, broad spectrum antibiotics, and immunosuppressive or antineoplastic therapies.

In contrast to *C. albicans, Cryptococcus neoformans,* the anamorph of *Filobasidiella neoformans,* is not usually a commensal of humans. *Cryptococcus neoformans,* a basidiomycetous yeast, includes two varieties that are often haploid when isolated from nature. *Cryptococcus neoformans* var. *neoformans* includes serotypes A, D, and AD, and *C. neoformans* var. *gatti* includes serotypes B and C. Serotypes A and D are usually associated with soil enriched with pigeon droppings. Serotype A is most common in the U.S.A. and serotype D is reported mostly from Europe. Serotypes B and C seem confined to warm regions (Kwon-Chung and Bennett 1984); in particular serotype B has been associated with the flowering of *Eucalyptus camaldulensis* (Ellis and Pfeiffer 1990). *Cryptococcus neoformans* most often primarily infects the lungs with mild symptoms. In stressed patients, a pneumococcal-type pneumonia is not uncommon. This species has a predilection for the central nervous system and may produce, if untreated, a fatal meningoencephalitis, particularly among the immunocompromised. Reporting of cryptococcosis is not mandatory, therefore its incidence is unknown. In Georgia, U.S.A. in 1978, Bowman and Ahearn noted 76 cases of cryptococcosis. Kaufman and Blumer (1978) estimated that there were about 300 cases of cryptococcal meningitis throughout the U.S.A. for that same year. In 1987, in Georgia, U.S.A. at least 146 infections occurred (Ahearn, unpublished). Throughout the U.S.A., there has been a notable increase in reported cryptococcosis. The Centers for Disease Control, Atlanta, Georgia, U.S.A., has documented cryptococcosis as the primary presenting syndrome for the acquired immune deficiency syndrome (AIDS) in 135 cases for 1983, 279 cases for 1984, 514 cases for 1985, and 704 cases for 1986 (R.M. Selik, personal communication). This steep increase involves fatal cryptococcal meningitis in 7–17% of patients with AIDS (Gal et al. 1986, Zuger et al. 1986). By 1990, cryptoccoccosis had become the fourth to fifth most important secondary infection complicating AIDS in the U.S.A. (R.M. Selik, personal communication). At least 2000 cases of extrapulmonary cryptococcosis (an incidence of about 7%) occurred among AIDS patients in the U.S.A. in 1990 (Centers for Disease Control 1991). Probably 450 more cases of cryptococcosis occurred among non-AIDS patients for this same year. The actual rate of increase of cryptococcosis over the past decade cannot be determined because not only is

reporting of the disease not mandatory, but the basis for defining AIDS, which is receiving intensive monitoring, has become less stringent during the past few years. Nevertheless, the worldwide increase in the incidence of life threatening cryptococcosis is alarming. Although oral candidiasis, and to some degree esophagitis, are prognostic of HIV infection (Phelan et al. 1987), systemic candidiasis has not shown the same relative increase among AIDS patients as has cryptococcosis.

Candida tropicalis is probably the third most important yeast pathogen of humans. This species does not normally produce germ tubes or chlamydoconidia, but elongated pseudohyphal cells may develop commonly in some strains in serum at 37°C. *Candida tropicalis,* like *C. albicans,* is a diploid ascomycetous-type yeast, but it is commonly isolated from host-free habitats. Both species have overlapping antigenic properties; *C. tropicalis* and *C. albicans* serotype A bear the mannan specific epitotes 1, 4, 5, 6, whereas *C. albicans* serotype B has 1, 4, 5, 13b (Tsuchiya et al. 1965). Hamajima et al. (1988) detected a cell wall antigen (t) in *C. tropicalis* that permits its serological distinction from *C. albicans* ser A. DNAs from the holotype strains of these species show insignificant DNA relatedness, but rare strains with phenotypic properties intermediate between those of the holotypes show intermediate (40–50%) relatedness with the separate species (Hamajima et al. 1988; Ahearn and Meyer, unpublished). Deep or systemic infections by *C. tropicalis* appear to be increasing and there is some suggestion that the prognosis for a disseminated infection with *C. tropicalis* is more grave than that for *C. albicans* (Ahearn and Lawrence 1984).

Because host factors in general are preeminent in yeast infections, it is difficult to determine specific virulence factors for many yeasts. Nevertheless, there appears to be an association of specific species with certain syndromes. In part, this may reflect a natural habitat association. For example, the usually commensal or mildly pathogenic, lipophilic *Malassezia* spp. may cause fungemia and fatal pulmonary infections of neonates receiving lipid supplementation via broviac catheters (Guého et al. 1987b, Danker et al. 1987, Larocco et al. 1988). Even the important industrial yeast, *Saccharomyces cerevisiae,* which can be expected to be ingested in normal diets, may produce fatal complications in immune deficient patients (Ossama et al. 1989; Aucott et al. 1990). Funguria or fungemia by skin mycota or commensals of mucus membranes (e.g., *Candida parapsilosis, Candida glabrata,* respectively) frequently follow combinations of extensive antibacterial therapy and catheterization (Solomon et al. 1984, Van Cutsem et al. 1986). In other instances, such as with the emerging pathogens *Trichosporon* spp., *Candida lusitaniae* and *Candida norvegensis*, the selective effect of antifungal therapies may be an additional factor (Ahearn and McGlohn 1984; Gargeya et al. 1990, Nielsen et al. 1990, Walsh et al. 1990).

Selected yeasts implicated as pathogens of humans and their common habitats are listed in Table 2. This list is restricted in comparison to general lists of medically important (or human- and animal-associated) yeasts because it includes only those species that fit the criteria of McGinnis et al. (1980):

- the fungus must be isolated;
- the fungus must be properly identified;
- the clinical disease must be compatible with a mycosis;
- the fungus must be seen in clinical and pathological specimens.

Species such as *Rhodotorula rubra, Candida famata, C. haemulonii, C. krusei, C. lipolytica,* and *C. rugosa,* which have been associated mainly with transient fungemia, have not been included. Undoubtedly, specific case histories in the future will indicate these species and others can produce a broader syndrome. For example, *C. haemulonii*, a genetically heterogenous form species complex, is more frequently becoming associated with lower limb ulcers and fungemia (Gargeya et al. 1991, Lehmann et al. 1991).

The increasing incidence of yeast infections has stimulated the development of commercial yeast identification systems (Land et al. 1991). Some of these systems provide for accurate identification of most strains of the common medically important yeasts, but generally the data bases for these systems include information for less than 50 species. Also, given strains may deviate from the norm in key characters. For example, *C. albicans* may not readily produce germ tubes, chlamydoconidia, or even pseudohyphae (Ahearn and Schlitzer 1984). Some strains of *Cryptococcus neoformans,* particularly *a* mating types, may produce melanin only under certain conditions (Nurudeen and Ahearn 1979); other rare strains may not demonstrate urease activity or assimilate inositol. Rhamnose may not be assimilated by rare strains of *C. lusitaniae* (Gargeya et al. 1990). An increasing number of auxotrophs of *C. albicans* and *C. glabrata* have been observed among yeasts submitted for identification to the Centers for Disease Control, Atlanta, GA. Such isolates, probably induced by the administration of antineoplastic drugs, do not grow on the generally used synthetic media, but often grow in the complex fermentation broths.

A selective phenotypic key for the identification of the more common adventitious yeast pathogens and some of the common animal-associated yeasts is presented below. Steps in the key include multiple characters, so that the key permits a presumptive species identification, i.e., a distinction of the adventitious species from most species presented as animal-associated in this monograph. For definitive identification, however, additional diagnostic tests must be performed. The key does not distinguish the genotypic species present in the form species *C. albicans, C. haemulonii, C. parapsilosis, C. tropicalis* and *Malassezia furfur.*

Table 2
Syndromes and habitats of yeasts causing infections in humans

Species	Representative infections		Habitats	
	Superficial	Deep	Association with humans	Host-free
Candida albicans	vaginitis	disseminated	mucus membranes	soil, air, water, plants (rare)
Candida glabrata	vaginitis (rare)	pyleonephritis, osteomyelitis	urine, sputa, feces	soil, water (rare)
Candida guilliermondii	otitis (rare)	endocarditis, joint infection	sputa, feces	soil, water
Candida kefyr (= *Kluyveromyces marxianus*)	unknown	disseminated	sputa	dairy products
Candida krusei	unknown	disseminated	sputa	water, soil
Candida lusitaniae	unknown	disseminated	sputa, feces	animal manure
Candida norvegensis (= *Pichia norvegensis*)	unknown	disseminated	sputa, urine	water, soil
Candida parapsilosis	onchomycosis (rare)	endocarditis, endophthalmitis	skin, sputa	soil, water, plants
Candida tropicalis	vaginitis (rare)	peritonitis, endocarditis	sputa, urine, feces	organically enriched soil and aquatic habitats
Candida viswanathii	unknown	meningitis	unknown	unknown
Cryptococcus neoformans	skin (rare)[a]	pulmonary, CNS	sputa (rare)	aged pigeon droppings, eucalyptus flowers
Malassezia furfur (M. pachydermatis)	dermatitis	pulmonary	skin	unknown
Pichia angusta	unknown	lymph nodes	unknown	insects, soil
Saccharomyces cerevisiae	unknown	disseminated	sputa	food (fruits), soil
Trichosporon spp.	white piedra	disseminated	skin, hair	soil, water

[a] Secondary manifestation of deep infection.

2. Key to selected human- and animal-associated yeasts

1. a Growth on Sabouraud's, malt agars, or yeast nitrogen base with glucose agar → 2
   b No growth on the above media without addition of olive oil .... *Malassezia furfur*: p. 782
   M. sympodialis: p. 783
   c No growth on yeast nitrogen base with glucose agar without olive oil, growth present on Sabouraud's or malt agars .... *Malassezia pachydermatis*: p. 782

2(1a). a Germ tubes produced .... *Candida albicans*: p. 476
   C. dubliniensis: p. 479
   b No germ tubes produced → 3

3(2b). a Hyphae or pseudohyphae absent or scarce → 4
   b Hyphae or pseudohyphae well developed → 16

4(3a). a Inositol assimilated (rarely negative), starch synthesized → 5
   b Inositol not assimilated, starch not synthesized → 9

5(4a). a Nitrate assimilated → 6
   b Nitrate not assimilated → 7

6(5a). a Sucrose assimilated .... *Cryptococcus albidus*: p. 748
   b Sucrose not assimilated .... *Cryptococcus terreus*: p. 764

7(5b). a Lactose assimilated .... *Cryptococcus laurentii*: p. 759
   b Lactose not assimilated → 8

8(7b). a Galactitol assimilated; melanin synthesized .... *Cryptococcus neoformans*: p. 762
   b Galactitol not assimilated; melanin not synthesized .... *Cryptococcus uniguttulatus*: p. 765

9(4b). a Cultures red or orange; nitrate not assimilated .... *Rhodotorula mucilaginosa*: p. 820
   b Cultures not red or orange; nitrate assimilated or not assimilated → 10

10(9b). a Nitrate assimilated → 11
   b Nitrate not assimilated → 12

11(10a). a Methanol assimilated .... *Pichia angusta*: p. 286
   b Methanol not assimilated .... *Pichia jadinii*: p. 314

12(10b). a Lactose assimilated .... *Candida famata*: p. 503
   b Lactose not assimilated → 13

13(12b). a D-Xylose and cellobiose assimilated (see also *C. famata* and *C. norvegensis*) ... *Candida guilliermondii*: p. 511
b D-Xylose assimilated; cellobiose not assimilated → 14
14(13b). a Galactitol assimilated ... *Candida haemulonii*: p. 511
b Galactitol not assimilated → 15
15(14b). a Raffinose assimilated and fermented ... *Saccharomyces cerevisiae*: p. 361
b Raffinose neither assimilated nor fermented ... *Candida glabrata*: p. 508
Candida norvegensis (*Pichia norvegensis*): p. 328
16(3b). a Arthroconidia produced → 17
b Arthroconidia not produced → 22
17(16a). a Diazonium Blue B (DBB) reaction positive → 18
b DBB reaction negative → 19
18(17a). a Nitrate assimilated ... *Trichosporon pullulans*: p. 869
b Nitrate not assimilated ... *Trichosporon ovoides* complex: p. 869
19(17b). a D-Xylose assimilated → 20
b D-Xylose not assimilated ... *Geotrichum capitatum*: p. 575
20(19a). a Cellobiose assimilated ... *Geotrichum fermentans*: p. 576
b Cellobiose not assimilated → 21
21(20b). a Arthroconidia in chains within primary filaments ... *Galactomyces geotrichum*: p. 210
b Arthroconidia in branched chains arising on branches from primary filaments ... *Geotrichum klebahnii*: p. 578
22(16b). a Lactose fermented ... *Candida kefyr* (*Kluyveromyces marxianus*): p. 236
b Lactose not fermented → 23
23(22b). a Raffinose and melibiose assimilated ... *Candida guilliermondii*: p. 511
b Raffinose and melibiose not assimilated → 24
24(23b). a Trehalose assimilated → 25
b Trehalose not assimilated ... *Candida krusei*: p. 519
25(24a). a Cellobiose assimilated → 26
b Cellobiose not assimilated → 28
26(25a). a L-Rhamnose assimilated (rarely negative) ... *Clavispora* (*Candida*) *lusitaniae*: p. 148
b L-Rhamnose not assimilated → 27
27(26b). a Triphenyl Tetrazolium Chloride (TTC) reaction positive ... *Candida tropicalis*: p. 563
b TTC reaction negative to weak ... *Candida viswanathii*: p. 570
28(25b). a Maltose fermented, TTC reaction positive or negative → 29
b Maltose not fermented, TTC reaction positive (rose to red color) ... *Candida parapsilosis*: p. 536
29(28a). a Sucrose fermented, TTC reaction positive (deep maroon color) ... *Candida tropicalis*: p. 563
b Sucrose not fermented, TTC reaction negative → 30
30(29b). a Maltose assimilated (repeat germ tube test) ... *Candida albicans*: p. 476
b Maltose not assimilated ... *Candida zeylanoides*: p. 571

Chapter 3

The industrial and agricultural significance of yeasts

A.L. Demain, H.J. Phaff and C.P. Kurtzman

Our ability to harness the activities of yeasts has given us not only food and drink but deeper insights into the chemical basis of our own existence. These microscopic organisms surely rank among the most fruitful and versatile of the domesticated plants.

(Paraphrased from Anthony N. Rose, 1960)

Contents

1. Introduction

Yeasts are used in many industrial processes, such as the production of alcoholic beverages, biomass (baker's, food, and fodder yeasts), and various metabolic products. The last category includes enzymes, vitamins, capsular polysaccharides, carotenoids, polyhydric alcohols, lipids, glycolipids, citric acid, ethanol, carbon dioxide, and compounds synthesized by the introduction of recombinant DNA into yeasts. Some of these products are produced commercially while others are potentially valuable in biotechnology. Some uses of yeasts in the food, beverage and fermentation industries (Jacobson and Jolly 1989) are shown in Table 3. The present review focuses on yeast species with current or potential value in biotechnology.

The use of yeasts dates back to ancient days. Before 7000 BC, beer was produced in Sumeria. Wine was made in Assyria in 3500 BC and ancient Rome had over 250 bakeries which were making leavened bread by 100 BC. Milk has been made into Kefyr and Koumiss using *Kluyveromyces* species in Asia for many centuries.

Yeasts are producers of the four leading fermentation products in terms of tons/year worldwide. These are beer (60 million tons), wine (30 million tons), single cell protein and fodder (800 000 tons), baker's yeast (600 000 tons). A fifth product is citric acid (500 000 tons) which is made by yeasts and molds.

2. *Saccharomyces cerevisiae* and other yeasts for bioethanol production

This group is represented by numerous strains that were chosen and adapted for specific industrial fermentations. They include baker's yeast (different strains for compressed and active dry yeast), wine yeasts (including special flocculent strains for the production of champagne and film-forming strains for the production of flor sherry), sake yeast, top and bottom fermenting brewing strains (varying in the degree of flocculation occurring during fermentation), and distiller's strains used for alcohol production from cereal starch. Strain improvement by genetic manipulation and protoplast fusion has contributed superior strains for the above processes. About two million tons of yeast are produced for the distilling, brewing and baking industries each year. The production of beverage alcohol is restricted to the use of microorganisms (e.g., yeast) but that of industrial and fuel alcohol was usually carried out by chemical synthesis from petroleum. This has changed in favor of yeasts. For example, in 1977, yeast production of beverage, industrial and fuel alcohol was 20% less than by chemical synthesis. By 1984, yeasts provided 87% more ethanol than did chemical synthesis. The percentage of total alcohol made by yeasts from sugars and starch has continued to increase.

Although hydrolyzates of plant materials such as cellulose and hemicellulose contain sugars not utilizable by *S. cerevisiae,* other yeasts such as *Candida lusitaniae* and *Pachysolen tannophilus* can convert cellobiose and xylose, respectively, to ethanol. Inulin from Jerusalem artichokes can be fermented to ethanol in concentrations of 5–7% v/v by *Kluyveromyces marxianus* (synonyms *K. fragilis, Candida kefyr, C. pseudotropicalis, C. macedoniensis), Torulaspora delbrueckii* (synonym *Saccharomyces fermentati*), *Debaryomyces occidentalis*

Table 3
Some present and potential uses of yeasts in the food, beverage and fermentation industries

Application	Yeast
Ale fermentation	*Saccharomyces cerevisiae*
Bread and dough leavening	*S. cerevisiae, Saccharomyces exiguus, "Saccharomyces rosei"*
D-Arabitol (sweetener)	*Candida diddensiae*
Emulsifier	*Candida lipolytica*
Ethanol fermentation	*S. cerevisiae*
Fish and poultry feeds (astaxanthin)	*Phaffia rhodozyma*
Fodder and single cell protein	*Candida utilis*
Lactose and milk fermentation	*Candida pseudotropicalis, "Kluyveromyces fragilis", Kluyveromyces lactis*
Lager beer fermentation	*"Saccharomyces carlsbergensis" (= S. pastorianus)*
Mannitol (humectant)	*"Torulopsis mannitofaciens"*
Shoyu, Miso	*Zygosaccharomyces rouxii*
Wine fermentation	*S. cerevisiae*
Xylitol (sweetener)	*"Torulopsis candida"*
D-Xylose fermentation	*Candida shehatae, Pachysolen tannophilus, Pichia stipitis, Pichia segobiensis*

(Schwanniomyces castellii) and *Torulopsis colliculosa* (= *Torulaspora delbrueckii*) (Guiraud et al. 1982).

3. Enzymes

Certain yeast enzymes have been extremely useful in industry. These include invertase from *K. marxianus, Saccharomyces carlsbergensis* (= *S. pastorianus*) and *S. cerevisiae* for candy and jam manufacture, β-galactosidase (lactase) from *K. marxianus* or *K. lactis* for production of syrups from milk or whey, and α-galactosidase from *S. pastorianus* for crystallization of beet sugar. Inulinase from various yeasts has the capability to produce fructose in high concentration from inulin in Jerusalem artichokes and chicory.

4. *Kluyveromyces marxianus* and related species

Several species of the genus *Kluyveromyces* are good lactose fermenters and are therefore useful in alcohol production from milk whey, an abundant byproduct of the cheese industry (Moulin and Galzy 1981, O'Leary et al. 1977). DNA hybridization experiments (Fuson et al. 1987) among the species recognized by van der Walt (1970c) have shown that DNAs from the type strains of *K. fragilis, K. bulgaricus, K. cicerisporus,* and *K. wikenii* exhibit between 90–100% complementarity with *K. marxianus* DNA. All are therefore synonyms of *K. marxianus,* which has priority. However, the lactose-fermenting ability of the strains varies from absent to strong, with "*K. fragilis*" strains (or its anamorph *Candida pseudotropicalis*) giving the strongest fermentation and thus being the most useful in whey fermentations.

5. *Candida utilis* for animal feed (fodder)

C. utilis is the most widely used species for the production of feed yeast in animal diets. Its ability to grow on cheap substrates, not suitable for most other yeasts, has made it the organism of choice for this purpose. *C. utilis* requires no added vitamins, can utilize ammonium or nitrate nitrogen, can utilize many carbon sources (including cellobiose and D-xylose), and grows at 37°C. Crude substrates, such as sulfite waste liquor (a byproduct of the paper industry) and wood hydrolyzate, are used for its propagation. *C. utilis* was formerly known as *Torulopsis utilis,* and the product is sometimes referred to as Torula yeast. The sexual state is known as *Pichia (Hansenula) jadinii* (Kurtzman et al. 1979, Manachini 1979).

6. Bioconversions

Yeasts can be used for a number of bioconversions (De Mot and Verachtert 1984). These include ketoreduction, oxidation of hydroxyl groups, ester hydrolysis and hydrogenation of double bonds in steroids; conversion of alkaloids and xenobiotics; L-lysine production from L-aminocaprolactam; L-phenylalanine from *trans*-cinnamic acid; conversions of *n*-tetradecene to 1-tetradecene, and phenol degradation.

7. Flavor compounds

A number of yeast products are useful in the flavor industry. Ethyl acetate and acetaldehyde are produced by *C. utilis.* Acetaldehyde is also formed by film yeast in the production of Spanish flor sherry (Phaff and Amerine 1979). Esters and other volatiles are made by *K. lactis* and monoterpenes are formed by *Ambrosiozyma monospora.* Terpenes and lactones are produced by *K. lactis, Torulaspora delbrueckii* and *Sporobolomyces odorus* (Drawert and Berger 1984). Citric acid, an acidulant which is also used by flavor companies, is made by various yeasts (see below) as well as the mold *Aspergillus niger.*

8. Yeasts capable of growing on hydrocarbons

A large number of yeast species are currently recognized for their ability to utilize hydrocarbons as sole sources of carbon and energy. Bos and de Bruyn (1973) listed 114 species and varieties of hydrocarbon-assimilating yeasts. Some of these have since been reduced to synonymy with other species. Species are found in the genera *Candida, Debaryomyces* (including *Schwanniomyces* and *Wingea*), *Lodderomyces, Clavispora, Metschnikowia, Pichia, Stephanoascus,* and *Yarrowia*; basidiomycetous species are in genera *Leucosporidium, Rhodosporidium, Sporidiobolus, Sporobolomyces, Rhodotorula,* and *Trichosporon.* All hydrocarbon-utilizing yeasts appear to have coenzyme Q-9 (Co Q-9) in the respiratory chain, but the reverse is not necessarily true, i.e., some yeasts with Co Q-9 do not assimilate hydrocarbons. No species of *Saccharomyces* and *Kluyveromyces,* all of which have Co Q-6, grow on hydrocarbons. Only straight-chain hydrocarbons (*n*-alkanes or *n*-alkenes) are utilized and those with 10–18 carbons are superior to those with shorter or longer chains. The rate of growth on hydrocarbons varies greatly. *Candida maltosa* (syn. *Candida cloacae, Candida subtropicalis*) (Meyer et al. 1975), *C. lipolytica* (sexual state = *Yarrowia lipolytica*), and *Candida tropicalis* (with many synonyms) (Barnett et al. 1990, Meyer et al. 1984, and the current volume) are among the best hydrocarbon utilizers. The metabolism of hydrocarbons by yeasts and the cell organelles involved has been reviewed briefly by Phaff (1985). The biotechnology industry has spent much money and effort in developing an economically feasible process for the production of single cell protein (SCP) from yeasts grown on *n*-alkanes (Tannenbaum and Wang 1975, Tuse 1984), but the industry experienced many disappointments. Thus SCP from hydrocarbons has virtually disappeared, partly due to economic factors, but mainly to failure to obtain approval for marketing the product from public health officials in some countries (Chen and Peppler 1978, Tuse 1984). Hydrocarbon-utilizing yeasts also play a significant role in the microbial oxidation of oil spills on land and in aquatic environments. Iizuka and coworkers (Iizuka and Goto 1965, Iizuka and Komagata 1965) described the microflora found in oil fields and oil brines in Japan, which included several hydrocarbonoclastic strains. Turner and Ahearn (1970) reported that hydrocarbon-utilizing yeasts increased more than 1000-fold after a spill of waste oils into a watershed pond. Bartha and Atlas (1977) reviewed in detail the role of microorganisms in stimulating biodegradation of aquatic oil spills, especially with help from dispersants to make the oil more accessible to microbial attack.

9. Methanol-utilizing yeasts

After the discovery in 1969 that some yeast species can grow on methanol (Ogata et al. 1969), interest in using methanol as a pure, water-miscible, and relatively inexpensive substrate for the production of SCP (Cooney and Levine 1975, Tuse 1984) increased rapidly. The metabolism of methanol by yeasts is different from that in bacteria, and its pathway has been elucidated by Quayle and coworkers (see Phaff 1985). Sahm (1977) listed several processes for the production of certain amino acids, citric acid, α-ketoglutarate, and hypoxanthine by methylotrophic yeasts. He reported that approximately 26 yeast species are known for their ability to grow on methanol as the sole carbon source. Some of these have been reduced to synonymy with other species and some more recently discovered species have been added (Barnett et al. 1990, and the current volume).

10. *Phaffia rhodozyma*

This pigmented, fermentative species has potential in biotechnology because its carotenoids are mainly astaxanthin (Andrewes et al. 1976), a pigment that is also responsible for the orange to pink color of salmonid flesh and the reddish color of boiled crustacean shells. Feeding of pen-reared salmonids with a diet containing this yeast induces pigmentation of the white muscle (Johnson et al. 1977, 1980b). Fish absorb the pigment better from cells that are mechanically disrupted than from intact cells (Tangerås and Slinde 1994). Enzymatic digestion of the cell wall of *P. rhodozyma* with a commercial multicomponent enzyme Novozym^{TM}234 from *Trichoderma harzianum* or with a β-glucanase complex from *Bacillus circulans* WL-12, greatly improves the extractibility and availability of the carotenoid in the diet of salmonids (Johnson et al. 1978, 1979, 1980b, Okagbue and Lewis 1983, Tangerås and Slinde 1994). Laying hens deposit astaxanthin in egg yolks when fed broken *P. rhodozyma* cells in their diet, but not with intact cells (Johnson et al. 1980a).

One problem with *P. rhodozyma* as a source of astaxanthin (as compared to synthetic astaxanthin) is its low pigment content in wild-type strains (~350 μg/g). Johnson and coworkers were able to raise the astaxanthin content of *P. rhodozyma* by a series of mutagenic treatments with antimycin A, resulting in yeast strains containing ~1500 μg of astaxanthin/g of yeast (An et al. 1989, 1991). Currently, at least two commercial companies have produced mutants of *P. rhodozyma* with astaxanthin contents of more than 3000 μg/g (Sanderson and Jolly 1994, Anonymous 1994). At this level of pigment content, the yeast-derived astaxanthin appears to be economically competitive with the synthetic product, which became available in 1984. Cultural conditions for *P. rhodozyma* are also important for highest astaxanthin titers. A low substrate concentration (<1.5%) throughout the growth cycle using the fed-batch technique and highly aerobic conditions are essential. Temperature for growth must be maintained at ~22°C (maximum growth temperature = 25–26°C) at which the doubling time is 4–5 h (Tangerås and Slinde 1994). Molasses appears to be an excellent

substrate for growth. The use of cellobiose as a carbon source also gave high astaxanthin yields in a wild-type strain (Johnson and Lewis 1979), but is uneconomical compared to molasses.

11. Riboflavin-producing yeasts and yeastlike organisms

Riboflavin (vitamin B_2) is not required for the growth of any yeast. All yeasts appear to produce enough riboflavin to satisfy their growth requirements, but a few are natural overproducers of this vitamin. This tendency of uncontrolled synthesis of riboflavin was found primarily in two species: *Eremothecium (Ashbya) gossypii* and *Eremothecium ashbyi.* Overproduction in natural strains of *E. ashbyi* was discovered by Guilliermond (1936) and in *E. gossypii* by Wickerham et al. (1946a). During the stationary phase of growth, the vacuoles become yellowish but, in some vacuoles, rosettes of needle-shaped crystals of riboflavin are observed. This has led to its long-time use for the industrial production of riboflavin in animal feed formulae (Perlman 1979). Later, *E. ashbyi* was replaced in industry by the more stable *E. gossypii*. The effects of composition of growth media and strain modification by mutation on riboflavin synthesis have been reviewed in detail by Demain (1972). Recently, Kurtzman (1995) placed *A. gossypii* in the genus *Eremothecium* based on ribosomal DNA sequence comparisons. Strains of *Candida famata* have been obtained which produce 20 g/liter of riboflavin by protoplast fusion and by mutations to resistance to the following: 2-deoxyglucose, ultraviolet, iron, the purine analogs tubercidin and 4-aminopyrazolo(3, 4d) pyrimidine, spent medium, and adenosine monophosphate (Foster et al. 1992, Heefner et al. 1992, 1993).

12. Citric acid-producing yeasts

The discovery in the late 1960s that very high yields of citric acid could be produced from *n*-alkanes by *Yarrowia lipolytica* caused this yeast to be competitive with *Aspergillus niger* (see Kapoor et al. 1982 for literature on this subject). Phaff (1985) reviewed the biochemistry and physiology of citric acid production by *Y. lipolytica.* Later studies have shown that citric acid can also be produced from vegetable oils or glucose under aerobic conditions by *Y. lipolytica, Candida zeylanoides,* and *C. citrica* (Kapoor et al. 1982, Phaff 1985).

13. Extracellular polysaccharide-producing yeasts

A large number of microbial species produce extracellular polysaccharides that can be recovered from growth media (Kang and Cottrell 1979, Slodki and Cadmus 1978). Most of the polysaccharides that have found industrial applications (e.g., xanthan gum) are produced by bacteria, but a few polymers from yeasts or yeastlike organisms have shown potential utility in foods or are used in industrial processes. The attractiveness of microbial polysaccharides is due in part to their differential stability and physical behavior in solution.

Several species of the genus *Pichia (Hansenula)* produce copious quantities of extracellular phosphomannans (Slodki and Cadmus 1978) from glucose under aerobic conditions. A strain of *P. capsulata* is able to convert 40–55% of the substrate into phosphomannans (Slodki and Cadmus 1978). In spite of attractive viscosity and gel properties for possible use as food additives, application of these water-soluble gums has been hampered because of their sensitivity to salts, shear, and heat, as well as their instability at low pH (Kang and Cottrell 1979).

A second example is the production of the glucan pullulan by strains of the dimorphic yeastlike fungus *Aureobasidium pullulans* (syn. *Pullularia pullulans*) often referred to as "black yeasts". Typical pullulans are linear polymers of predominantly repeating units of maltotriose connected by α-(1 $\rightarrow$ 6)-bonds, with molecular weights ranging from 1×10^4 to 4×10^5 (Kang and Cottrell 1979). The induced biosynthesis of pullulans has been reviewed by Slodki and Cadmus (1978). Fermentation yields of pullulan from starch hydrolyzates in 10% concentration are as high as 75%. Kang and Cottrell (1979) reviewed the proposed and patented applications of pullulans, which include use as a flocculator of clay slimes in hydrometallurgical processes, in coating and packing material for foodstuffs and pharmaceuticals, in adhesives, and in the manufacture of special fibers and fabrics.

14. Additional applications

A number of additional activities carried out by yeasts may have industrial or environmental significance. These include degradation of paraquat and diquat by *Lipomyces starkeyi,* depolymerization of tannin extracts by *Zygosaccharomyces (Saccharomyces) rouxii,* ascorbic acid production from galactose by *Candida norvegensis,* formation of α-carotene by *Rhodotorula glutinis* and L-phenylalanine production by *Rhodotorula rubra.*

15. Yeasts as hosts for expression of recombinant DNA

The introduction and expression of heterologous (foreign) genes in yeasts serves as an alternative to bacteria for the expression of foreign polypeptides. *S. cerevisiae* is not ordinarily pathogenic to humans (see chapter 2) and thus foreign proteins synthesized in this yeast may be more acceptable in pharmaceuticals and in food products for human consumption. Additional advantages that can be attributed to *S. cerevisiae* are: (i) the high stability of its cells; thus, there is little or no lysis under various adverse conditions of growth. (ii) Defined growth media used for yeasts contain few extracellular protein species under ordinary growth conditions and provide good conditions for secretion studies and recovery of gene products of recombinant DNA. (iii) Yeasts, in contrast to most bacteria, can glycosylate proteins. Glycosylation in

recombinant yeast of normally non-glycosylated bacterial proteins results in more stable proteins. (iv) *S. cerevisiae* has had a long history of use in industrial fermentations. (v) Yeasts are much more able than *Escherichia coli* to secrete heterologous proteins into the extracellular medium.

The procedure is to insert a heterologous gene in a plasmid capable of replication and selection in yeasts. Also needed is a 5′-flanking DNA promoter sequence to increase transcription. These have been derived by various investigators from the 5′-flanking sequences of genes such as yeast alcohol dehydrogenase 1, yeast glyceraldehyde-3P-dehydrogenase, 3-phosphoglycerate kinase, α-factor, or acid phosphatase. A transcription terminator must be present in the 3′-flanking fragment.

To obtain relatively pure gene products, it is desirable that they are secreted into the growth medium by the yeast. It has been found that translocation of secretory proteins (such as yeast invertase and acid phosphatase) across the endoplasmic reticulum involves the recognition and cleavage of a hydrophobic amino-terminal extension, the so-called signal sequence. Schekman and Novick (1982) showed that the secretion pathways in yeasts and mammalian exocrine cells are similar. After translation of the preprotein at the endoplasmic reticulum, the signal peptide is removed after passage through the membrane, and the processed protein is then transported to the Golgi apparatus where vesicles are formed which transport the protein to the cytoplasmic membrane of the bud. Fusion of the vesicles with the membrane releases protein from the cell, either in the periplasmic space of the cell envelope or into the medium. Because of the ubiquitous nature of the signal sequences in different eukaryotic secretory proteins, yeast enzymes involved in the processing and secretion have been found to work also with foreign gene products. A large number of heterologous proteins are secreted by yeasts using the yeast mating factor (MFα1) signal sequence, i.e., the prepro-α-factor directed system.

The following are examples of some heterologous genes cloned in *S. cerevisiae* and secreted by the recombinant yeast: human interferons (Hitzeman et al. 1982, 1983), wheat α-amylase (Rothstein et al. 1984), human epidermal growth factor (Brake et al. 1984), human interleukin-α, calcitonin, human growth hormone-releasing factor, human serum albumin, human insulin-like growth factors, prochymosin, insulin and somatostatin (Brake et al. 1984). Human interferon-γ is produced at 5–10% of total cell protein (Egan and Bitter 1984) and human hemoglobin continuing endogenous heme at 2–3% of total protein (Wagenbach et al. 1991). Human superoxide dismutase is made in stationary phase cells at a remarkable level: 70% of total cell protein (Hallewell et al. 1987). The sweet-tasting plant protein thaumatin is synthesized in yeast with plasmids encoding preprothaumatin (Edens et al. 1984). Although the signal sequence was found to be indispensable for thaumatin synthesis in yeast, the protein (which is about 2000 times sweeter than sucrose on a weight basis) was not secreted into the growth medium. Similarly chymosin, a proteinase found in the fourth stomach of unweaned calves and used commercially in the first stages of cheese making, is not secreted by *S. cerevisiae* carrying the cloned genes of the various forms of this protease (Mellor et al. 1983). The zymogen, prochymosin, is inactive but becomes activated by an autocatalytic process at low pH. Calf prochymosin was synthesized to over 5% of total yeast cell protein. Bacterial proteins have also been synthesized and processed in yeast as exemplified by β-lactamase (Breunig et al. 1982) and the *E. coli* outer membrane protein A (Janowicz et al. 1982). The most important commercial yeast recombinant process has been the incorporation of the genes encoding surface antigens of the hepatitis B virus resulting in the first safe hepatitis B vaccine (Wilson 1984).

The methylotrophic yeasts, *Pichia pastoris* and *Pichia angusta (Hansenula polymorpha),* have some advantages over *S. cerevisiae* as hosts for heterologous genes. (i) These yeasts can be grown at extremely high cell densities (100–130 g dry weight per liter) in protein-free media. (ii) They have a higher level of protein productivity. (iii) In contrast to hyperglycosylation by *S. cerevisiae,* the methanol-utilizing yeasts do not overglycosylate. (iv) Foreign genes are incorporated in multiple copies into the chromosomes of methylotrophic yeasts. (v) The levels of production by these yeasts are relatively high (Table 4). The *P. pastoris* system (Brierly et al. 1990) employs a methanol-regulated alcohol oxidase gene promoter which yields alcohol oxidase at 30% of soluble protein. The expression cassette is stably integrated into the host genome at specific locations. The organism is haploid and amenable to traditional mutagenesis.

Table 4
Recombinant proteins produced by methylotrophic yeasts

Protein	Level (g/l)
Bacterial streptokinase	0.3
Epidermal growth factor-like protein	0.5
Bovine lysozyme (secreted)	0.6
Hepatitis B surface antigen	1.0
Glucoamylase (secreted)	1.4
Hirudin (secreted)	1.5
S. cerevisiae invertase (secreted)	3.0
Tumor necrosis factor	10.0

16. Improvement of yeasts by mutation, protoplast fusion and recombinant DNA methodology

Because of the conservatism of the food industry, many improvements in yeasts via construction of new strains by recombinant DNA methods (see below) have not yet been commercialized. However, mutation and protoplast fusion methods have been used to transfer genetic characteristics because this methodology does not come under

the heading of recombinant DNA techniques. Mutation of *Saccharomyces diastaticus* (= amylolytic strains of *S. cerevisiae*) to 2-deoxyglucose resistance resulted in increased rates of wort fermentation and unchanged beer flavor (Stewart et al. 1985). Mutation of *Candida boidinii* to ethionine-resistance increased methionine content from 0.5–9 mg/g dry cells in the intracellular amino acid pool and from 6–16 mg/g in the cells themselves (Tani et al. 1988). Some of the genetic characters transferred from one yeast to another by protoplast fusion are (i) osmotolerance (from osmotolerant yeasts to the food yeasts *S. cerevisiae* and "*S. diastaticus*", (ii) flocculation (Panchal et al. 1982), (iii) lactose utilization (Farahnak et al. 1986), (iv) the "killer" character (Bortol et al. 1986), (v) cellobiose utilization (Pina et al. 1986), and (vi) methionine overproduction (Brigidi et al. 1988).

With regard to recombinant DNA techniques, the endoglucanase gene has been transferred from the mold *Trichoderma reesei* into brewer's yeast for hydrolysis of barley β-glucans, thus decreasing haze in beer and improving filtrability (Penttilä et al. 1987). Brewer's yeast has also been the recipient of the amyloglucosidase gene from *Aspergillus niger* for hydrolysis of unfermentable dextrins in production of light beer (Hammond 1988). The same gene from *Aspergillus awamori* (Cole et al. 1988) and the mouse pancreatic α-amylase gene have been incorporated into distiller's yeast allowing ethanol production directly from starch. Brewer's yeast containing the α-acetolactate decarboxylase gene from *Enterobacter aerogenes* no longer makes diacetyl and produces beer with a markedly reduced maturation time (Sone et al. 1988). Lower acidity and enhanced flavor in wine has been achieved by transformation of wine yeast with the gene encoding the malolactic conversion enzyme from *Lactobacillus delbrueckii.* Plant α-galactosidase, introduced into *S. cerevisiae,* allows the yeast to modify guar gum to achieve the desireable properties of locust bean gum. In 1990, a recombinant baker's yeast containing heterologous genes encoding melibiase and maltose permease was approved in the U.K. for breadmaking but has not yet been used, presumably due to fear of consumer response.

17. Yeasts as plant pathogens

Yeasts are generally not known to be plant pathogens, but members of the genus *Eremothecium* (synonyms *Ashbya, Holleya, Nematospora*) are exceptions. As discussed earlier, two species of this genus, *E. ashbyi* and *E. gossypii,* are used for commercial production of the vitamin riboflavin.

Batra (1973) provided a thorough review of the host range for many of the Eremotheciaceae and it was noted that infections are usually insect-vectored. *Eremothecium cymbalariae, E. ashbyi* and *E. gossypii* commonly infect cotton (*Gossypium* spp.) as well as the fruits of *Citrus* spp. Infections often take the form of surface lesions, especially on fruits, but the fibers from infected cotton bolls may show yellow discoloration as well. Other economically important plants that are infected by these three species include coffee (*Coffea* spp.) and *Hibiscus* spp. *Eremothecium coryli* has a wider host range than the preceding species and commonly infects cotton, tomato (*Lycopersicum esculentum* Mill), *Citrus* spp., soybean (*Glycine max* (L.) Merr.) and hazel nuts (*Corylus avellana* L.). In contrast, infections caused by *E. sinecaudii* are restricted to seeds of oriental mustard (*Brassica juncea* (L.) Coss.) and yellow mustard (*B. hirta* Moench) (Holley et al. 1984).

18. Biological control of post-harvest diseases of fruits and grains

Rotting of stored fruits, vegetables, and grains following harvest represents a significant loss to agriculture, but control of rotting through chemical treatment may introduce health and environmental risks. An alternate strategy to prevent fungal decay relies on surface treatment of stored crops with bacteria and yeasts antagonistic to fungi (Wilson and Wisniewski 1989). Yeasts are favored over bacteria because they do not produce antibiotics and they are perceived as less likely to cause human disease when consumed.

Droby et al. (1989) and Chalutz and Wilson (1990) reported that citrus fruits treated with a strain of *Debaryomyces hansenii,* which was reidentified as *Pichia guilliermondii* (McLaughlin et al. 1990), were protected from common mold-induced rots. Protection appeared to result from competition for nutrients because *P. guilliermondii* did not inhibit the molds (*Penicillium digitatum* (Pers. ex Fr.) Sacc. and *P. italicum* Wehmer) when it was cocultivated with them on laboratory media or when the molds were exposed to filter-sterilized media following growth of *P. guilliermondii.* Similar results were obtained by Roberts (1990), who reported control of gray mold (*Botrytis cinerea* Pers. ex Pers.) disease of apples following treatment with living cultures of *Cryptococcus laurentii.*

The use of yeasts to prevent molding of stored grains shows some promise. Paster et al. (1993) demonstrated that growth of *Aspergillus flavus* Link:Fr. on soybeans was significantly inhibited when the seeds were coinoculated with *Pichia guilliermondii.* Björnberg and Schnürer (1993) showed *Pichia anomala* to inhibit the grain storage molds *Penicillium roqueforti* Thom and *Aspergillus candidus* Link when grown together on laboratory media. Inhibition apparently did not result from competition for nutrients.

19. Food and beverage spoilage yeasts

Spoilage of foods and beverages from growth of contaminating yeasts results in major economic losses worldwide. The extensive literature on this subject precludes a thorough treatment in this book. Further information can

be found in Phaff et al. (1978a, pp. 224–237), Pitt and Hocking (1985), and Fleet (1990).

Briefly, undesirable changes caused by yeasts in fresh and processed foods, or during and after their fermentation, may consist of pellicles or turbidity in liquid products or as a slimy or powdery coating on solid products, causing the formation of off-odors and flavors. Species responsible for food spoilage are not known to cause food poisoning as is the case with certain bacteria.

Yeasts responsible for food spoilage are usually well-defined and even predictable species, which depend on the chemical composition of the product, its pH, temperature of storage and the presence or absence of oxygen. Improperly cleaned processing equipment is often the source of contamination. Yeasts occurring in products with a high sugar content (40–70% by weight) grow slowly, especially at the higher concentrations, and are usually represented by species of the genus *Zygosaccharomyces* or by *Torulaspora delbrueckii, Schizosaccharomyces octosporus, Pichia subpelliculosa* and a few other species. If a high osmotic pressure is created by the addition of NaCl as a preservative, different yeast species develop, depending on the salt concentration. During the lactic acid fermentation of Spanish style olives, the salt concentration is ~6.5% and *Pichia membranifaciens* and other *Pichia* species predominate. During the fermentation of cucumbers (salt concentration 10–16%), *Candida etchellsii, C. versatilis* and some other species develop. The highest salt concentration (to nearly saturation) favors the development of species of the genus *Debaryomyces,* which are found in aged cheeses, dry salami and similar meat products. Spoilage of fresh fruits (e.g., grapes, pears, tomatoes) is commonly caused by *Candida stellata, Pichia kluyveri, P. fermentans, Metschnikowia pulcherrima* and species of *Hanseniaspora* and its anamorph *Kloeckera.* Species of *Brettanomyces* (sexual state *Dekkera*) may cause turbidity and off-flavors in wines, beer, and soft drinks. Yeasts associated with dairy products are usually endowed with lactase activity (*Kluyveromyces marxianus, K. lactis,* as well as some *Rhodotorula* species). Yeasts with lipolytic enzymes *(Yarrowia lipolytica, Candida oleophila)* are found in triglyceride emulsions (e.g., olive pulp), while those containing amylases (species of *Saccharomycopsis*) can cause spoilage of starchy foods.

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Chapter 4

Ecology and yeasts

Marc-André Lachance and William T. Starmer

Contents

1. Introduction

Louis Pasteur, Emil Christian Hansen and Martinus Beijerinck figure prominently among early students of yeast ecology. Although educated as a chemist, Pasteur was the first to put forward the notion that microorganisms are essential in the global turnover of living matter, and that yeasts are necessary components of the microbiota of fermenting wine or beer by effecting the conversion of sugar to ethanol. According to Dubos (1976), Pasteur's ecological thinking was a major determinant of the success of his endeavors. Hansen, recognized as the founder of yeast systematics, provided the first insights on the distribution of yeasts in their natural habitats (Phaff et al. 1978a). Beijerinck described his own work as "microbial ecology", years before the coming of age of that science (van Iterson et al. 1940). Better known for his creative uses of bacterial enrichment techniques and his studies of geochemical cycling, Beijerinck also made important contributions to yeast ecology, including studies on the resistance of yeast ascospores to physical stresses, and the application of this phenomenon to the isolation of yeasts from soil samples.

Because ecology is the study of any aspect of organisms in the context of their environment, all investigations of yeast morphology, physiology, or genetics, be they descriptive or predictive, may be spiced with an ecological flavor. Yeast ecology has, therefore, progressed largely in parallel with the development of studies of yeast taxonomy and physiology by workers who may not have viewed themselves as ecologists. Many aspects of these developments have been reviewed comprehensively by Phaff et al. (1978a) and by various contributors to volumes 1 and 2 of Rose and Harrison's (1987) treatise.

Ecology often addresses the study of life from many of the same perspectives as evolutionary biology, but on a different time scale. Whereas evolutionary responses involve changes in gene frequencies over generations, ecological adaptation may occur repeatedly during the life of an organism and does not usually involve changes in the genotype of that organism. At the interface of these two sciences, evolutionary ecology examines the interaction between the genetic profile of organisms as members of communities interacting with their environment. This relatively new approach had its first major manifestation in yeast ecology when a multidisciplinary congregation of researchers combined efforts to examine the interdependence of cacti, yeasts, and drosophilids. The early results of this research were brought together in a monograph by Barker and Starmer (1982).

Because the impact of human activity on nature is overwhelming, yeast ecology can be of economic or political importance. Often overlooked in the past was the potential role of yeasts in environmental ecology, specifically as agents of pollution, bioremediation, or biological pest control. Of course, some yeasts are well known as the central components of some industrial processes, which may in fact constitute complex microbial ecosystems. The theoretical framework underlying the selection of industrial yeast strains from the standpoint of known or presumed selective forces acting upon yeasts in nature has been dealt with by Lachance (1990b).

This chapter, rather than paraphrasing the publications cited above, will focus on yeast ecology in general as an art and a science, on its possible future developments, and on some connected topics of current interest.

2. Taxonomic descriptions are the basis for yeast ecology

As Phaff et al. (1978a) stated in conclusion to a general discussion of yeast ecology, "we are convinced that imaginative research by yeast ecologists will lead to the discovery of many additional interesting associations and the isolation of novel yeast species." This prophetic view has been substantiated by the spectacular growth in the number of known yeast species from 341 in Lodder's 1970 edition of *The Yeasts, a Taxonomic Study* to 597 in the 1990 monograph of Barnett et al. (1990), in spite of the substantial number of synonymies elucidated by molecular taxonomists in the interval. Unfortunately too many times in the past, workers engaged in yeast isolation have not gone beyond the mere nomenclatural description of new

species, failing to typify also their community, habitat, and possible interactions. A significant number of species listed in taxonomic monographs are known only through a single strain whose origin is vaguely described.

In view of the rapid decline of many natural habitats due to urban and industrial development of the few remaining wilderness areas worldwide, the need to search for new yeasts is pressing. The urgency of the problem as it applies to all forms of life has been expressed by E.O. Wilson: "I think a world biological survey would do more for humanity during the next 20 years than the genome mapping project" (Horgan 1994). The increased awareness, among world leaders, of the importance of documenting biodiversity allows one to be filled with optimism about the future of taxonomic research. However, it is vital that new taxonomic surveys be couched in an ecological framework, and that isolation methods be devised to yield ecological insights. The alternative is a meaningless catalogue of names, devoid of any real biological relevance. A tangible risk exists that such an inventory could even be used as an excuse to justify further destruction of natural habitat.

3. Yeast community, niche, or habitat: some definitions

Yeasts do not occur randomly throughout the biosphere. They form communities of species. Each community may be defined by its habitat, which is the actual place where an assemblage of yeasts lives, and by the niches of its component species. The niche consists of the attributes that make a yeast capable of sharing a habitat with other members of the community. The niche is thus the sum of all physical, chemical, or biotic factors required for successful existence. The members of a community may have different niches that happen to overlap in a given habitat. It is important to distinguish, among species found in a habitat, those that are essential components of the community from those that are transient or present fortuitously. The former are termed autochthonous, and the latter allochthonous. In practical terms, when evidence exists that particular yeast species actually grow regularly in a substrate, the species may be considered autochthonous members of a community. If that substrate repeatedly yields the same small array of species, it is considered to be a highly specific habitat. Likewise, yeast species endowed with a broad niche and as a consequence occupy many habitats are regarded as generalists. Other yeasts only occur in very unique habitats, and thus are considered specialists. It goes without saying that the one time observation of a yeast species in a substrate does not allow one to draw any such conclusions.

3.1. The yeast niche

By their very nature, yeasts are generally limited in the range of habitats they can occupy. In addition to many mineral nutrients that serve as building blocks for most of their cell constituents, yeasts require significant amounts of an organic source of carbon and energy of relatively small molecular weight. The high surface/volume ratio of yeasts favors rapid nutrient absorption. Their unicellular nature often makes them better suited for deep liquid substrates or moist and uneven surfaces, unlike molds, which form hyphae that can penetrate and liquefy semisolid substrates or spread over smooth, inert surfaces. The size and shape of yeast cells may be a major factor in the ease and selectivity with which they are vectored by certain insects, and the volatiles that they produce may serve as specific attractants.

Yeasts usually grow over a broad range of pH values, allowing them in particular to colonize materials that have already been the site of fermentative activities by bacteria. Conversely, yeasts are not truly thermophilic. A maximum growth temperature of 46–48°C is observed in the intestinal species *Arxiozyma (Saccharomyces) telluris* and related taxa (Watson 1987). Some yeasts (e.g., *Pichia* and *Debaryomyces* spp.) possess hydrophobic wall components that cause them to form a film at the surface of liquids, while others (e.g., *Saccharomyces* or *Hanseniaspora* spp.) may have the ability to form dense flocs. These features define the niche of yeasts, and in turn allow us to predict that yeast habitats will most often be rich in simple organic carbon, liquid or very high in moisture, acidic or occasionally alkaline, and nutritionally complex. Such conditions are met by plant tissue undergoing various forms of decay, as well as exudates of roots, leaves, or flowers. Some yeasts may be better adapted to conditions met in association with the body of certain animals, usually acting as intestinal commensals.

It is useful to distinguish between the intrinsic attributes that affect the potential distribution of yeasts from the sum of all factors that actually determine their effective distribution. The terms *fundamental* versus *realized* niche are used to specify those two aspects of the niche (McNaughton and Wolf 1979). Although one generally expects the two to go hand in hand, Starmer (1981a) deduced a negative correlation between the fundamental and realized niches of certain cactophilic yeasts, based on the observation that some nutritionally specialized species had a broader host range than some nutritional generalists. The latter may be an exception. Cosmopolitan yeast species such as members of the genera *Debaryomyces* and *Cryptococcus* are most heterogeneous in their nutritional abilities, whereas yeasts that have predictable, specialized habitats, for example species of *Saccharomyces, Pichia,* or *Hanseniaspora*, exhibit narrower nutritional potentials. At the extreme, the yeast *Cyniclomyces guttulatus* possesses probably the narrowest known fundamental niche, being defined by unusually stringent nutritional requirements and a limited range of growth temperatures and redox potentials. The realized niche of *C. guttulatus* is further restricted by the coprophagous behavior of its host, the rabbit.

3.2. The yeast habitat

Gams (1992) stated that saprophytic microfungi behave according to the principle that "everything is everywhere, the environment selects." He incidentally attributes the well-known phrase to Bass-Becking, and not to Beijerinck, as is thought by many (Atlas and Bartha 1993). Notwithstanding, a literal adhesion to this postulate could lead one to believe that with enough persistence, it might be possible to isolate any yeast almost anywhere. The opposite extreme would be to limit the search for yeasts entirely to known yeast habitats, with the consequence that many new species could be missed altogether. Between these two absurd positions, the middle ground is the conduct of taxonomic studies within an ecologically sensible framework. The discovery of new yeast habitats may be serendipitous in some cases and guided by previous knowledge in others. Regardless, once yeast populations have been located for study, an effort should be made to identify their actual habitat, which in turn will generate an appreciation for the actual community. For example, many yeasts found in the sea may not always be autochthonous components of a seawater community. A search for the primary habitat, although not easily accomplished, is desirable. Many new yeast species, some of which are represented by a single strain, have been recovered from soil after some kind of selective treatment to eliminate molds and bacteria. Since soil is the ultimate repository of all life forms, any effort aimed at testing whether soil isolates are in fact autochthonous to soil would be worthwhile.

3.2.1. Aquatic habitats: Fresh and estuarine waters are difficult to characterize as specific yeast habitats, given that their microbiology is highly affected by the surrounding terrestrial fauna and flora, soil run-off, or effluents of human sources. In their review of this topic, Hagler and Ahearn (1987) noted the predominance, in fresh water habitats, of yeasts normally considered ubiquitous or associated with pollution. Vincent (1988) noted that the basidiomycetous yeasts recovered in Antarctic lakes were clearly the result of wash-in from adjacent soils. Moreover, polar soil yeasts, which occur in significant numbers, were found mostly in soil samples that "also contained moss, lichen or microalgal material". The reported isolations would gain a more profound significance if the actual yeast habitats were identified at the source.

Sieburth (1979) observed that yeasts are the dominant fungi in the oceans, although they are outnumbered by bacteria. Whereas basidiomycetes appear ubiquitous, ascomycetous yeasts seem more responsive to the concentration of organic debris. Among ascomycetes, the generalistic *Debaryomyces* species abound, specially near shores. Also frequently isolated are *Metschnikowia* species known to be associated primarily with marine invertebrates such as calanoid copepods, with fish, or with algae (Fell 1976). Up until recently, *Kluyveromyces aestuarii* had been isolated exclusively, albeit sporadically, from the sea. This was of interest, because (according to our laboratory records) that species is significantly more resistant to sodium chloride than other members of the genus. Recent work by Hagler's group (de Araujo et al. 1995) has demonstrated the abundance of *K. aestuarii* in crabs, which could account entirely for its scattered occurrence in the sea.

The mostly psychrophilic *Leucosporidium* spp., *Rhodosporidium* spp., *Candida austromarina, Candida natalensis,* and *Sympodiomyces parvus* are probably autochthonous marine species, since they are recovered at relatively high cell concentrations from open ocean samples in specific circumpolar locations (Fell 1976). These are not usually isolated elsewhere. According to Fell (1976), marine yeasts are best defined by their combined ability to survive for long periods in seawater and to utilize a broad array of carbon compounds. Differential responses to salt are not likely to be significant. Because this broad definition might also apply to many generalistic yeasts, it remains of interest to wonder if some yeasts are intrinsically marine by virtue of specific physiological characteristics, such as psychrophily. MacLeod (1965) defined marine bacteria as having a highly specific need for sodium cations, in some cases a requirement for halide ions, and a higher dependence on magnesium and calcium than terrestrial bacteria. He remarked that contrary to common belief, the halophily and psychrophily of many bacteria isolated in the sea were not genetically stable. The essential feature of marine bacteria is their ability not only to survive, but also to grow in the sea, which ostensibly involves complex physiological attributes. A large proportion of marine bacteria grow poorly if at all on laboratory media. Whether the same criteria can be extended to yeasts is questionable. Hagler and Ahearn (1987) remarked that dilute media such as water or soil extract agar are of no advantage in isolating yeasts of marine origin, unlike for many marine bacteria.

As is the case for freshwater yeasts, many yeasts found in the sea may be allochthonous. Many are species of *Rhodotorula* or *Sporobolomyces* or other phylloplane genera (Hagler and Ahearn 1987) and thus marine isolates of these yeasts may originate from plant foliage. The frequent isolation of *D. hansenii* in seawater is consistent with its general ubiquity. Yeasts found both in the sea and in fresh water are most certainly allochthonous. They include *Candida* species thought to originate from fecal contamination, and some *Cryptococcus* species also found in soils and on plant surfaces.

3.2.2. Terrestrial habitats: Terrestrial yeasts are most abundant in plants, animals, and soil. This is a very broad topic which was reviewed extensively by Phaff and Starmer (1987), and so only a few special issues will be raised here. As mentioned above, the recognition of soil as a yeast habitat is problematic. In terms of overall abundance, it would appear that filamentous fungi tend to outnumber yeasts in most soils (Gams 1992). Nonetheless,

as outlined by Phaff and Starmer (1987), the existence of yeasts that are isolated repeatedly and exclusively from the soil, such as *Lipomyces* species, *Debaryomyces (Schwanniomyces) occidentalis, Schizoblastosporion* sp., and certain species of *Cryptococcus* suggests that some habitat specificity may be at play. Vishniac (1994) demonstrated that *Cryptococcus albidus*, a dominant soil organism, was capable of rapid growth when introduced into autoclaved soil, following which viability was retained for 2 months. She argued that the sterilization treatment altered the nutritional value of the soil in a manner similar to natural weathering factors. Consistent with this, the growth of indigenous soil yeasts would be a function of the frequency and intensity of disturbances in soil.

Terrestrial ecosystems in general are the most amenable to studies of yeasts which participate in complex relationships with other organisms. Atlas and Bartha (1993) compiled an extensive list of possible community interactions as follows. Microbe–microbe interactions include neutralism (sparse independent populations sharing the same habitat), commensalism (a population benefits from the activity of another), synergism (cooperative modification of resources leading to close spatial relationships), mutualism or symbiosis (very strong synergistic association), competition (utilization of limited common resources), amensalism (chemical interference), predation (ingestion), and parasitism (long term destructive contact). Plant–microbe interactions are defined by the terms rhizosphere and rhizoplane, mycorrhizae (mutualistic), phyllosphere and phylloplane, epiphytes, and pathogens. Animal–microbe interactions encompass grazing, food enrichment, and epizoic relationships. Several of these have been documented in yeasts (Phaff and Starmer 1987). Some examples will be given below.

Starmer et al. (1991) summarized the major factors that can shape complex yeast communities, using as an example the cactus–yeast–*Drosophila* system. The first factor, dispersal, can be highly specific as it may involve insects that feed only on certain cactus species. Drosophilids are grazers that inoculate specific yeasts into injured plants. The yeast community developing in the cactus phyllosphere may become fashioned by the action of toxic compounds present in the host tissue (plant–microbe amensalism). Specifically, cacti in the genus *Stenocereus* produce saponins, some of which are toxic for many yeasts. Moreover, yeasts (and bacteria) already present in the host tissue may interact with newly introduced species (microbe–microbe interactions). In some cases, the interaction is mutualistic, and in others, it may involve competition for nutrients (Starmer and Fogleman 1986). Amensalism is also suspected in cases where species exclusion may be correlated with their reciprocal responses to killer toxins (Starmer et al. 1987). Last, the yeasts present in the resulting assemblage must provide sufficient benefits (food enrichment, detoxification) to the vector for the latter to carry on further dispersal of the community to a new host.

Evidence that killer toxins play an ecologically significant role as a form of amensalism involving different yeasts is still limited. Yeasts collected from decaying fruits are generally more likely to be killers than yeasts from other communities such as tree fluxes or cactus rots (Starmer et al. 1987). Cross-classification of yeasts from within and between communities demonstrated more killing activity between communities. This would suggest that yeasts within a community have adapted to their respective toxins, and that killer toxins may act to exclude yeasts from other communities. Moreover, Ganter and Starmer (1992) found that the killer activity of cactophilic yeasts was dependent on the geographic region, the host plant, and the habitat from which the yeasts were collected.

Many naturally selected wine yeasts are killers (Young 1987). This phenomenon is widespread, but evidence that killer toxins actually serve as an instrument of competition between yeast strains in wine is only starting to accumulate (Carrau et al. 1993). Brewery contaminants capable of taking over from cultured strains and of causing bad flavors are usually killers, whereas non-killing contaminants are of lesser consequence (Young 1987). Conversely, a recent study of a natural tequila fermentation suggests that killing is not a significant factor in the development of a stable yeast community in that case (Lachance 1994).

Ultimately, the composition of a yeast community depends on the niche compatibility of its member species. Autochthonous components may interact together in a synergistic manner or cohabit in a neutral relationship due to resource partitioning. Non-members may be excluded by various forms of competition, or simply remain present, but as allochthonous elements due to the unsuitable nature of their fundamental niche.

4. Yeasts in the food chain

The role of yeasts in geochemical cycling is probably negligible in comparison to that of bacteria. On the other hand, it is now quite clear that yeasts frequently fulfill an important role in the food chain. Insects not only feed on substrates known to serve as yeast habitats, but also exhibit several adaptations to yeast utilization as a feedstock. Since the beginning of the 20th century, yeasts have been recognized as important and essential components of drosophilid nutrition (Delcourt and Guyénot 1910, Baumberger 1919). In addition, the importance of yeasts may go well beyond mere nutrition. Recent research indicates that yeasts may affect drosophilids by (1) effecting the detoxification of chemicals that are harmful to larvae or adults (Starmer 1982b, Starmer et al. 1986), (2) producing cues used by larvae or adults to find food (Fogleman et al. 1981, 1982), (3) signalling the location of egg deposition sites (Vacek et al. 1985, Barker

et al. 1986), (4) altering pheromones released by adults to attract mates (R.J. Bartelt, personal communication), and (5) influencing the behavior of adults during courtship and mating (Cook and Cook 1975, McRobert 1986, Starmer et al. 1988b).

The principal function fulfilled by ascomycetous yeasts as insect food appears to be the conversion of mineral or other low complexity nitrogenous compounds to a rich mixture of proteins and other complex nutrients beneficial for the insects. It is less clear whether basidiomycetous yeasts occupy a similar position along the hierarchy of trophic levels. Clearly their utilization of phylloplane and cauloplane exudates could cause a nutritional enrichment of these habitats in favor of grazing invertebrates. In the sea, yeasts may serve as food for filter feeding animals. However, the amount of yeast biomass on plant surfaces or in the sea is presumably much lower than that found in the deeper layers of the phyllosphere.

5. Characterizing yeast communities

5.1. Yeast isolation

Because yeasts hardly ever occur naturally as pure cultures, a balance must be struck between the wish to identify all components regardless of abundance and the need to recognize which components are most significant. In this respect, direct streak plating of suitably diluted substrate (with a calibrated loop) offers the advantage that it preserves the abundance ratios of different yeast types. YM agar is preferable to simpler formulations such as malt extract, which is poor in certain nutrients. We favor acidification to pH 3.7 over the addition of antibiotics to inhibit bacterial growth completely. Some laboratories opt for the incorporation of chloramphenicol (50–100 mg l^{-1}). If interference occurs between fast and slow growing species on the same plate, selective agar media can be formulated, and the sample plated in replicate. Natural substrates that yield intractable mixtures of yeasts and molds that must be treated by selective filtration, with or without liquid enrichment, may not actually harbour a genuine community of autochthonous yeasts. In cases where they do, enrichment methods can and should be altered to yield a quantitative assessment of yeast occurrence (e.g., most probable number techniques). A search for the true habitat may be more productive, in the long run, than the devising of complex isolation procedures. The isolation of rare ascosporogenous yeasts in soil can be facilitated by the use of Beijerinck's enrichment (van Iterson et al. 1940), which consists of subjecting soil to gradual desiccation and heating, with periodic sampling and inoculation of the soil. It is not clear how the method could be modified to generate a sense of the community to which those yeasts belong.

5.2. Sample size

Once a habitat has been identified and suitable methods designed, a collection scheme should be formulated to yield sufficient numbers of samples. Sampling strategies have been the object of numerous publications in general ecology, but not in yeast ecology. We (Lachance and Starmer 1986) have determined empirically that a minimum of 15 independent samples is required to obtain an accurate reflection of community composition for cactus necroses, insects, tree exudates, or flowers. The number varies according to the frequency of empty samples. If empty samples are present, the sampling size should be corrected to obtain a minimum of 8 non-empty samples.

Higher sampling intensities are sometimes useful if minor community components are sought. For example, the yeast *Sporopachydermia quercuum* occurs in low numbers (and presumably at low frequencies) in red oaks of the Great Lakes area. In an attempt to obtain a larger number of isolates to study variation (J.E. Kaden and M.A. Lachance, unpublished results), 50 trees were sampled and their exudates plated in duplicate on YM agar and Yeast Nitrogen Base agar with *myo*-inositol as sole carbon source, plus 10 mg l^{-1} cycloheximide. Only five separate isolates were obtained. At another locality, 20 trees were sampled and only one isolate was recovered.

5.3. Purification

The art of picking colonies for further study is an important aspect of community description. The isolation plates should be examined daily until the colonies acquire a sufficient size to allow discriminating between the types. It is generally advantageous to use some kind of magnifying device. High power (4×) reading glasses are convenient and suitable. A representative of each colony type should be recovered, and the number of colonies of this type estimated. We have found little advantage to picking replicates of each colony type unless the mating type ratios of a haploid heterothallic species are being investigated. The possibility that a species is missed because its colonies are identical to others is compensated by processing an adequate number of samples. Ideally, each colony should be immediately suspended in water and streaked out for purification. When this is not possible, colonies may be transferred directly to small (1–3 ml) agar slants and purified later.

5.4. Identification

Yeast identification methods have been standardized to some degree since the keystone publication of Wickerham (1951). The details, however, vary from one laboratory to another. For ecological studies, we favor replica plating with a multipoint inoculator (see Lachance 1987, for a description) for several reasons. Many ecological insights emerge from examining numerous yeast strains for growth on the broadest possible variety of media, whether or not all media are required for species identification. The number of informative tests may approach 90, and a typical field study may yield over 300 isolates. Simple arithmetic will show how onerous is the task of conducting these tests in separate tubes. We inoculate 21 strains

per plate. The preparation of enough media for 8 series of 90 media takes less than two days. One series requires about 2 hours to inoculate and 1 hour to read. Importantly, colony characteristics may vary from one medium to another, which may in itself constitute valuable information. Contamination is more easily detected on agar media than in broth. Special shaking equipment is not required. Replica plating has also some drawbacks such as cross-reactivity. For example, the urease hydrolysis test cannot be performed on replica plates, and tests for the assimilation of certain sugars (i.e., sucrose, raffinose) are sometimes difficult to assess correctly. These weaknesses of replica plating are largely outweighed by the advantages.

Complete identification of every isolate is essential. The use of simplified characterization procedures may be permissible in studies of already well characterized communities (Davenport 1980a), but in exploratory studies, "an accurate picture of yeast habitats cannot be assembled if the yeast species cannot be correctly identified" (Phaff and Starmer 1980). As newer molecular identification methods develop, one can easily conceive of the possibility that a primary isolation plate, a microscope slide, or even a fixed section of a yeast habitat could be treated with a mixture of DNA probes, each tagged with a different chromophore, in such a way that direct identification of individual colonies or even single cells *in situ* would be achievable. Nonetheless, the determination of a yeast's physiological abilities will never cease to be of importance in understanding its ecological niche.

Exhaustive physiological characterization is useful in identifying intraspecific variation. The taxonomist's search for stable characters is foiled by the fact that phenotypic variation is an intrinsic feature of evolving species. Strain variation, which may represent an annoyance in practical taxonomy, is an important element in the recognition that yeast populations in certain communities may constitute distinct biotypes subject to unusual forms of selection. The regularly advanced proposals to disregard completely various characters because they vary within a species should be resisted if taxonomy is to retain an ecological significance. In fact, if taxonomy is to preserve any biological relevance at all, taxonomists should not lose sight of the fact that in variation lies the very essence of biology.

5.5. Community definition

The description of a yeast community can range from a simple list of the species and their frequencies to a complex series of ecological parameters. Various measures of diversity based on the species names and frequencies are available (Atlas and Bartha 1993). We have found a quantity termed the effective number of species (Starmer 1982a) usually adequate and conceptually simpler than most entropy based expressions. Other workers (P. Morais, personal communication) have preferred the use of a related quantity termed the "odds" measure of diversity (Kvalseth 1991). Both measures are based on the probability of re-isolation of a species.

It may also be interesting to describe communities as the sum total of the phenotypic properties of their components. Again various statistics are available in the general ecological literature. We have used vectors of the mean results of identification tests to define yeast communities associated with different plant families (Lachance and Starmer 1982) and formulated a number of descriptors based on these vectors adjusted in reference to expected identification results (Lachance and Starmer 1986). This allows one to define a community on the basis of a higher or lower than expected performance on each test. For example, cactophilic yeasts constitute a highly specific community characterized by their significantly less than expected growth on most tested carbon sources, and more than expected growth at 37°C, whereas yeasts isolated from exudates of trees in the birch family, while less specific, exhibit a broader than expected carbon compound utilization and no significant departure from expectation vis-à-vis 37°C growth.

5.6. Analysis

Collections of yeast species and their frequencies, as well as their responses to identification tests, can be analyzed in the form of structured tables, in which similar communities and similarly distributed species are juxtaposed. Species frequencies are also eminently suitable for visualization by means of multivariate methods. A basic understanding of the assumptions and properties of relevant methods should be secured by prospective users, but the procedures, although complex, are easily executed using algorithms available commercially, through internet, or on most institutional computers. A comprehensive discussion of multivariate methods can be found in Legendre and Legendre (1983). The following discussion will be limited to some basic hierarchic clustering and ordination methods.

Clustering is most often performed with the UPGMA algorithm, which results in the erection of a dendrogram depicting the various degrees of similarity between communities. Users should be keenly aware that every clustering method imposes distortions, sometimes severe, upon the real structure of the data being analyzed. UPGMA engenders less distortion than many other clustering algorithms, but it will produce a hierarchic dendrogram no matter whether a hierarchic structure exists in the data or not. Clustering should therefore be used with a very critical eye and in conjunction with other methods.

Ordination allows complex data to be reduced to a small number of variables (usually 2) whose coordinates are plotted on a graph. Because multivariate data are multidimensional, reduction to two sets of coordinates will inevitably result in some misrepresentation of reality. A most useful ordination method in species-based community ecology is correspondence analysis (CA), because it is designed for the analysis of frequency tables.

Habitats and the species they contain can be plotted on the same graph on the basis of coordinates that represent the most significant departures from expectation. Species that cluster strongly with a particular habitat may represent a distinct community. Another ordination method, non-metric multidimensional scaling (MDS), creates a plot intended to represent the distance matrix describing communities according to species composition or any other descriptors. Although MDS does not allow the superimposition of species and communities on the same graph, it is applicable to any kind of data and achieves the same purpose as cluster analysis, but without creating a hierarchy that may not exist. A more widely known ordination method, principal components analysis, extracts from the data information elements that exhibit the highest amount of internal correlation. Each resulting component contains information that is uncorrelated with the information in the next. PCA should be restricted to normally distributed data, which are less frequently available in yeast ecology. However, in many cases, this analytical method may help generate insight from large sets of data even if many of its mathematical constraints are not followed to the letter.

6. Molecular ecology

6.1. Molecular identification of biotypes

Characterization of DNA sequences that are strain-specific allows the study of biotype distributions in yeast communities. This approach has been used to study natural yeast populations, but has received most attention in the control of implantation and contamination of commercial yeast strains in industrial fermentations. The simplest strategy is the production of strain-specific electrophoregrams by restriction endonuclease digestion of whole genome DNA. This has been used by Lachance (1990a) to study the global dispersal of a cactophilic yeast, *Clavispora opuntiae*. In that and many other species, tandemly repeated ribosomal DNA is present in such excess that simple agarose electrophoresis of restriction digests produces a clear rDNA banding pattern against a background of other sequences. The pattern can be used as a fingerprint or transformed into a map of the gene cluster. Most of the variation is due to spacer polymorphisms.

Electrokaryotyping can also yield unique patterns capable of differentiating strain genotypes. This has been applied successfully to yeasts of oenological (Vézinhet et al. 1990) and clinical (Iwaguchi et al. 1990) interest.

A further refinement to molecular identification is the use of labelled DNA probes applied to DNA transferred from a gel to a membrane. Probes are selected from highly variable regions of the genome (Degré et al. 1989) or designed arbitrarily (Walmsley et al. 1989).

A variation on the same theme is the use of arbitrarily designed probes to amplify polymorphic DNA fragments randomly by PCR (RAPD, Hadrys et al. 1992). The fragments are separated by electrophoresis. Because of its "black box" nature, RAPD is mostly useful at the preliminary levels of an ecological study, and should give way to other, less ambiguous methods when the community being studied becomes better understood.

As DNA sequencing technology progresses, it is quite clear that the complete sequence of suitably chosen DNA markers will constitute the most valuable sort of fingerprinting data. This requires the identification of hypervariable sequences of manageable size, flanked by more highly conserved regions that serve to design primers for amplification. Numerous methods presently exist to perform PCR amplification directly on a few yeast cells. In some laboratories, sequencing reactions are performed directly on amplification products in a few minutes. As rapid, non-isotopic methods for visualizing sequencing gels develop further, it should become possible to obtain a short (e.g., up to 300 bp) sequence from a few yeast cells in less than a day.

6.2. Molecular species tracking

Future developments in yeast ecology will no doubt include the application of molecular methods to the tracking of yeast species or individual lineages in their natural habitat. One such approach is the use of reporter genes to study the fate of introduced yeast clones into a habitat, or the movement of genes within natural yeast populations. One of the most promising is the bioluminescence (lux) gene cluster (Kado 1992).

Species-specific DNA probes also have much potential in this area. Whereas the technology that would allow exact *in situ* localization of individual yeast cells or colonies is still under development, the detection of designated species in small samples is now reality. Fell (1993) used a 3-primer system whereby DNA extracted directly from a dilute cell population is subjected to PCR. Each reaction contains two universal primers designed to generate a moderately sized rDNA fragment in any yeast. A third primer, specific to a chosen species, causes instead the appearance of a fragment that is approximately half as large if that species is present. With the rapidly progressing PCR technology, it is conceivable that the presence or absence of a dozen designated species can be assessed within one or two hours from the time the sample is recovered.

7. Ecological aspects of yeast sexuality

Theoreticians dealing mostly with organisms for whom reproduction is either exclusively or predominantly sexual have formulated numerous hypotheses regarding the evolutionary significance of sexuality (Michod and Levin 1988), some of which have become part of the biological canon, but none of which is entirely satisfactory. Andrews (1993) examined sexuality as a source of ecologically significant variation, contrasting microorganisms with complex eukaryotes. In differentiated multicellular organisms, the profound specialization opposing germinal

and somatic tissues has the important result that the environment only indirectly affects gene transmission, by influencing the reproductive output of each individual. In organisms capable of clonal reproduction, different ramets (clones) of the same genet (genetic individual) may transmit genetic variation acquired by mutation or other means to the next generation. Microorganisms are similar to clonal organisms, and thus their evolution is presumed to be less affected by sexuality in the strict sense and more by other factors. In the specific case of the yeasts, Andrews (1993) proposed that the Ty and mating type elements of *Saccharomyces* could represent potentially important sources of genetic variation.

Clonal reproduction notwithstanding, the frequent occurrence of heterothallic sexuality in haploid yeasts suggests that the sexual mating process is adaptive for those yeasts. However, the considerable success afforded to yeasts by their non-sexual reproduction makes it difficult to assess the effect of sexuality upon their fitness through ecological time. This is further complicated by the frequent occurrence of homothallism. We still do not know how extensive sexual reproduction is in natural yeast populations. A general tendency exists for heterothallic haploid yeasts to exhibit local imbalances in their mating-type ratios, which suggests that sexual mating is in fact rare, at least in ecological time. Lachance et al. (1994) examined this question from various angles in *Clavispora opuntiae* and observed that mating-type imbalances may be self-amplifying because the mating process itself is hindered by imbalances at the population level. Put differently, mating is maximum in an equal mixture of cells of the two mating types, and in turn, frequent mating would be an important factor in maintaining an even ratio of mating types. In that and several other yeasts, G_1 arrest is prerequisite to mating, and the arrest is often triggered by nitrogen starvation. It would then appear that ecological pressures favor the elimination of sexual reproduction in haploid yeasts, but that significant evolutionary forces are at work to maintain the process. Future research on the determinants of senescence in eukaryotic microorganisms (Andrews 1993) may provide elements towards understanding the nature of the selective forces involved in the maintenance of sexuality in yeasts.

More easily explained in ecological terms is the occurrence of haploid homothallic yeasts, specially those which form fewer than four ascospores, e.g., certain species of *Nadsonia, Debaryomyces, Citeromyces, Torulaspora, Hanseniaspora,* and others. Homothallism does not preclude cross-fertilization and consequently may well be responding to the same evolutionary pressures as other forms of sexuality. At the ecological level, however, the use of the sexual process to form one or two dormant spores which may exhibit accrued resistance to environmental stress is not only advantageous, but also economical, since it makes use of mechanisms that are already in place in the cell for its sexual functions. The fact that genetic recombination is not necessarily achieved in this case becomes unimportant.

If sexuality occurs freely enough in yeast populations, the various alleles in that population should be distributed randomly with respect to one another except in cases of genetic or selective linkage. Strong allele associations (linkage disequilibria) involving genes that are not linked physically can also be an indication of quasi-exclusively asexual reproduction in populations subject to significant fluctuations in size. Little work has been done in that area.

Cactophilic yeasts exhibit a wide diversity of sexual life histories. At one extreme, the common cactus yeast *Candida sonorensis* is always asexual wherever it has been collected throughout the world (i.e., four different continents). Yeasts classified as *Sporopachydermia cereana* are in fact a mixture of cryptic species, some of which are self-fertile, some asexual, and some which exhibit what appears to be a pheromone-mediated interaction that could be interpreted as vestigial sexuality. *Pichia cactophila,* another very common cactus yeast, occurs in nature in the homothallic and diploid state. *Pichia heedii* is heterothallic, but occurs mostly in the diploid state because its asci are persistent, which causes the fusion of sister spores of opposite mating types. The heterothallic *Pichia amethionina* and its close relative *Pichia caribaea* occur in nature in both the haploid and diploid states, and the spores are released early from the asci. *Pichia opuntiae* and the related saguaro-specific *Pichia thermotolerans* occur as haploids in their habitats. As mentioned earlier, *Clavispora opuntiae* exhibits clear-cut heterothallism and is predominantly haploid. The reasons for the differences in modes of sexual reproduction in these yeasts are not entirely clear, especially because some of them occur side by side in the same habitat (e.g., *P. heedii* and *P. thermotolerans*) and are subject to the same selective pressures.

8. Clinical ecology and the yeast connection

A relatively new ecological interest in yeasts stems from the proposal by Truss (1978) that *Candida albicans* may be responsible for numerous persistent ailments, ranging from chronic fatigue to depression. Termed "The Yeast Syndrome" (Trowbridge and Walker 1986), the condition allegedly results from three major activities of intestinal populations of *C. albicans*. One is the conversion of sugars to acetaldehyde; another is a disruption of the integrity of the intestinal lining, allowing large immunogenic molecules to enter the bloodstream; last, the problem is thought to be exacerbated by the release of a large number of antigenic and toxic substances by the yeast.

The belief that systemic candidiasis is a major factor associated in some way with a wide number of debilitating conditions, including schizophrenia, memory loss, sexual impotence, multiple sclerosis, rheumatoid arthritis, or chronic fatigue is attributed to Crook (1986), a proponent

of so-called clinical ecology, which is also known by some as "orthomolecular medicine". *Candida albicans* is even claimed to produce, in some cases, enough alcohol to result in chronic drunkenness, although at least one attempt to use this as a defense by a person charged with impaired driving has not borne fruit. The issue is complicated by the fact that diagnosis of systemic candidiasis typically does not require evidence that yeast is present in any significant amounts in the host, but is based on indirect evidence, including some rather obscure blood tests.

An integrated treatment is proposed, entailing oral intake of ketoconazole, caprylic acid, numerous "natural" preparations including lactic acid bacteria and garlic, vitamins including vitamin C, minerals, essential fatty acids, or massive doses of nystatin. Vaccination and other less obvious forms of immune desensitisation may also be advocated, as well as intravaginal applications of yogurt, or enemas with coffee or other preparations (Trowbridge and Walker 1986). The use of antibacterial antibiotics and steroid medication is discouraged. An unusual diet which eliminates anything thought to be connected with fungi is also advocated. The prohibited foods include sugars, breads, cheeses, alcohol, vinegar, malt and other grains, processed meats, mushrooms, and many nuts and fruits. Eggs, most vegetables, and most lean meats are allowed. Treatment must often be prolonged over several months or even years. The more drastic remedies can apparently trigger a major response of discomfort, nausea, or fatigue in patients. Termed "yeast die-off" or "Herxheimer reaction", the condition is attributed not to the possible side effects of antifungals, but instead to the toxicity of substances released by the killed yeast.

Given the extensive popularity and the anecdotal nature of alternative medicine, it is difficult to evaluate the well-foundedness of this new aspect of yeast ecology. It would be presumptuous and unfair to reject broadly all claims that many idiopathic conditions may be aggravated or even stimulated by *Candida albicans,* and most insensitive to dismiss the suffering of many as inconsequential. Conventional medicine remains sceptical (Bennett 1990), because of the refusal of clinical ecologists to approach their treatment scientifically, and the negative evidence obtained in controlled clinical trials. It is clear that systemic infections by *Candida albicans* are widespread and very serious (Edwards 1991) as a complication in patients whose health is critically compromised. It is less clear whether chronic candidiasis is one of the most prevalent and debilitating conditions afflicting modern humanity.

9. Yeasts as probiotics

In sharp contrast to the claim that ingestion of non-pathogenic yeasts, their byproducts, or those of other microorganisms might act as predisposing factors in systemic candidiasis, a growing body of evidence suggests that some yeasts may have significant beneficial effects. Indeed, the administration of baker's or brewer's yeast to patients suffering from diarrhea caused by *Clostridium difficile* has been shown, in clinical tests, to improve recovery (Schellenberg et al. 1994, Chia et al. 1995).

A commercial preparation made up of viable cells of *"Saccharomyces boulardii"* (possibly a biotype of *S. cerevisiae*) is effective in the treatment of various intestinal disorders, including chronic diarrhea in humans receiving antibiotics or nasogastric alimentation and in AIDS patients (McFarland and Bernasconi 1993). The treatment requires the presence of a sustained population of this yeast in the intestine, and has no significant effect on the normal intestinal microbiota. Upon cessation of the treatment, the yeast is eliminated in 2 or 3 days. Pathogens successfully antagonized by *S. boulardii* in gnotobiotic animals include, in addition to *Clostridium difficile, Candida albicans, Vibrio cholerae* and *Entamoeba histolytica*. The activity on *C. albicans* includes a decrease in the abundance of pathogenic yeast translocated to the lymph nodes. Although the mechanisms by which yeasts inhibit microbial pathogenic action are not completely understood, it appears that the cell wall glucans play a role in the detoxification and reduction of antigenicity of bacterial effectors.

10. Yeasts and microbial ecology

When asked why he had not pursued a lifetime research career in the study of yeasts, a prominent contemporary bacterial ecologist replied that "yeasts do not have an ecology". An extensive treatise entitled "Microbial ecology, principles, methods, and applications" (Levin et al. 1992) was published recently in response to the growing importance of the environmental role of microorganisms, in particular those used for biotechnological purposes. Although the yeast genera *Saccharomyces, Candida,* and *Kluyveromyces* are listed in the first chapter as having a potentially significant impact on human activity, no further mention of these or any other yeasts is made throughout the book. References to yeasts are also conspicuously rare in other major microbial ecology treatises (Andrews 1993, Atlas and Bartha 1993, Stolp 1988). Such a "poor cousin" figure is neither unexpected nor alarming, if it is realized that to a large degree yeasts play a rather minor role, in the biosphere, compared with other microorganisms that may act as primary producers, predators, pathogens, or important agents of nutrient cycling.

The role of yeasts in geochemical cycling takes second place to that of bacteria. As carbon cyclers, yeasts respire and often perform a fermentative glycolysis, but are rather restricted in the nature of the carbon sources they may assimilate. Few if any yeasts excrete cellulases or chitinases, thus restricting their contribution to decay. Extracellular amylases, lactases, maltases, proteinases, ureases, lipases, or other enzymes produced by yeasts are usually dwarfed by more powerful counterparts in

the bacteria or the filamentous fungi. The contribution of yeasts to nitrogen cycling sometimes involves nitrate reduction or ammonification of nitrite, but is usually restricted to the assimilation of ammonia or amino acids into organic nitrogen. Most yeasts act as sulfate reducers, and some are sulfur auxotrophs. Although many yeasts can grow at pH values as low as 2.5, none can challenge *Thiobacillus*, which is capable of growth at pH values near 0. The growth of *Debaryomyces hansenii* in 3.0 M sodium chloride is no rival to that of halophilic bacteria in saturated (5.2 M) salt, and no yeast could conceivably survive alongside thermophilic bacteria able to grow at 100°C. The list of examples of how meagre is the potential of yeasts to colonize extreme environments could go on for pages, but the point has undeniably been made that our preoccupation with yeast ecology cannot be enkindled by the same incitements as the bacteriologist's quest for more and more unusual habitats and for examples of organisms responsible for geologically significant phenomena. Instead, the study of yeasts in an ecological context has to address phenomena in which yeasts do participate.

In spite of the profound difference between yeasts and bacteria, yeasts lend themselves to most of the methodologies so useful in the study of bacteria. As a result, microbial ecologists with a bacteriological leaning may try to identify in yeasts patterns that may not apply to yeasts. At the same time, plant and animal ecologists, accustomed to observations based on more immediate identifications of their target organisms, often do not integrate microbial components in their otherwise powerful models. Andrews (1993) addressed this matter by drawing an extensive comparison of the ecologies of large and small organisms. Among the main fundamental differences he identified between these two kinds of organisms, the capacity of most microorganisms for extensive dormancy, high growth rates, narrow permissible growth conditions, a high degree of metabolic adaptability, the direct exposure of unicellular forms to selection, large numbers, a predominantly haploid condition, and a diminished role for sexuality. Not unexpectedly, most of the comparisons drawn by Andrews were directed at microorganisms other than yeasts.

We are convinced that yeasts can and should be studied extensively in an ecological and evolutionary perspective, and that such studies will yield bounteous rewards. Yeasts were a major focus of microbial ecology at very early stages, and could represent the organisms of choice to tackle the new challenges facing evolutionary ecology today. Moreover, future progress in yeast taxonomy should benefit greatly from accentuating its ecological flavor. But the nearly obligate association between yeasts and other living organisms will make it advantageous to use a multidisciplinary approach, and consequently yeast ecologists will gain by joining forces with specialists of other biological disciplines to pursue common endeavors.

Part III

Ultrastructural and molecular properties used for yeast classification

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Chapter 5

Cytology and ultrastructure of yeasts and yeastlike fungi

Royall T. Moore

Contents

1. Introduction

Classification of fungi is based extensively on morphology, mostly as determined by light optics. During the past 30 years, however, electron microscopy has provided critical new observations that have enlarged this foundation to include micromorphology (see Hohl 1987, Moore 1989a, 1994, 1996a). This new information, and that provided by molecular biology (Bruns et al. 1992), has sharpened and clarified the delimitations of a number of taxa, including the distinction between ascomycetes and basidiomycetes. One consequence of this expanded knowledge has been the general recognition of the Fungi as a kingdom on a par with those of plants and animals (Moore 1996c); another aspect has been the challenge of adapting the nomenclatural framework to reflect the additional taxonomic information (Moore 1974, 1994).

The kingdom Fungi is eukaryotic and characterized by cell walls that have a major chitin component (Bartnicki-Garcia and Lippman 1989), hyphae that extend apically (Wessels 1986, Bartnicki-Garcia et al. 1989, Howard 1981, Kotov and Reshetnikov 1990) and divide by centripetal invagination of the plasma membrane (see Moore 1985 and below). In general, fungal nuclei are haploid, small (~1 μm in diameter), have envelope pore complexes similar to those of other eukaryotes (Allen and Douglas 1990, Carter 1978, Nehrbass et al. 1990), and amounts of chromosomal DNA intermediate between that of bacteria and plants and animals (Sparrow et al. 1972, Durán and Gray 1989). Microtubular organizing centers (MTOCs) called spindle pole bodies (SPBs), chromosomes, and modes of nuclear division are unique. There is a single SPB (Fig. 1) intimately associated with the nuclear envelope (Heath 1981, O'Donnell and McLaughlin 1984a, Rout and Kilmartin 1990, O'Donnell 1992) that divides during nuclear division. Mitotic chromosomes are difficult to discern by light microscopy and they rarely become organized into a metaphase plate (see King and Hyams 1982, Burns 1988). In most species, the nuclear envelope persists during both

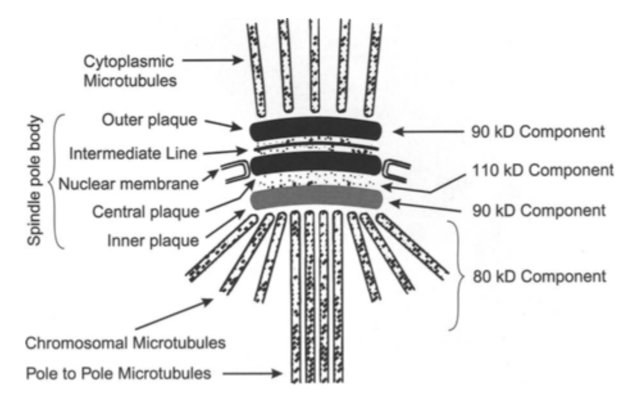


Fig. 1. Spindle pole body (SPB). SPB localizations and spindle components (from Rout and Kilmartin 1990).

mitosis (Boekhout and Linnemans 1982, King and Hyams 1982, Heath et al. 1982, Bourett and McLaughlin 1986, Tanaka and Kanbe 1986) and meiosis (Howard and Moore 1970, O'Donnell and McLaughlin 1984a). During nuclear division, the SPB divides and each SPB generates spindle and astral microtubules (mt's) as they migrate around the nuclear envelope to opposing polar positions (Heath 1981, O'Donnell 1992).

2. Cellular growth

2.1. Introduction

Yeasts, for the most part, are single-celled, typically uninucleate fungi that reproduce by forming new cells serially. The nature of the cell wall provides the initial dichotomy in classification. The difference in wall structure between ascomycetous and basidiomycetous forms is indicated by the electron microscopic image of the budding site. Ascomycetes (Fig. 2) have two closely appressed wall layers (that of the emerging bud and that of the parent cell) whereas for Basidiomycetes (Fig. 3), the parent cell wall is multi-layered and ruptured, appearing as a collar consisting of a series of splayed wall layers (Kreger-van Rij and Veenhuis 1971a, Miller et al. 1976b, Simmons and Ahearn 1987). Biochemical traits parallel this dichotomy: (1) ascomycetes give no visible response to diazonium blue B (DBB) while those of basidiomycetes give a reddish to purple color (van der Walt and Hopsu-Havu 1976, Hagler and Ahearn 1981, Simmons and Ahearn 1987); (2) only ascomycetous species give a positive response to certain protoplast releasing enzymes (Bastide et al. 1979, 1986); and (3) pyrophosphatidic acid

(pyro-PA) is found exclusively in basidiomycetous species (Goto et al. 1988).

Studies that examine changes during specific times in the cell cycle of yeasts and other fungi (Herskowitz 1988, Nasmyth 1996) frequently use cell division cycle (cdc) mutants. Four phases are commonly recognized in the cdc: G_1 – gap 1, an interval before DNA synthesis; S – an interval of DNA synthesis; G_2 – gap 2, an interval between the end of DNA synthesis and the initiation of mitosis; and M – mitosis.

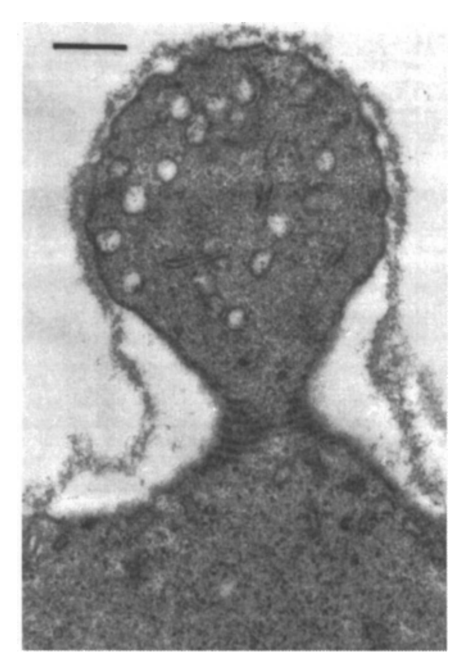


Fig. 2. Ascomycete-type budding *(Saccharomyces cerevisiae)*. Grazing section of an early bud showing a number of rings in the neck region (from Byers and Goetsch 1976). Bar = 250 nm.

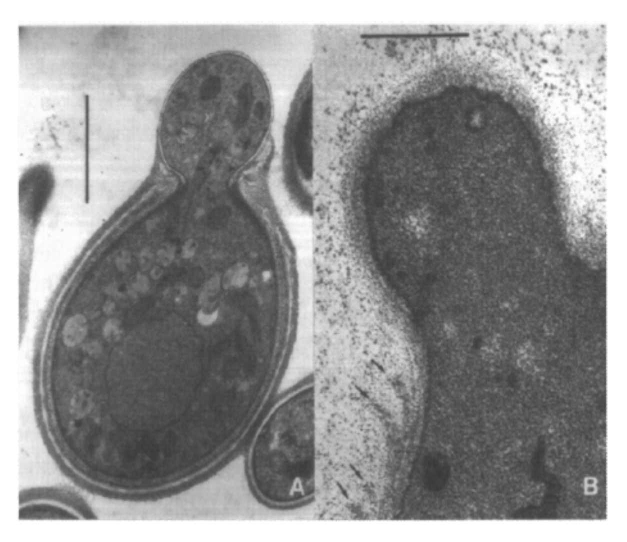


Fig. 3. Basidiomycete-type budding: (A) initial bud cell of *Filobasidiella (Cryptococcus) neoformans* (courtesy of R. Calderone, from Pelczar and Chan 1981). The dark:light:dark wall layers of the parent cell are prominent and are discontinuous at the budding site. The wall layers of the emergent bud are discrete from those of the parent cell and consist mostly of a shell of light material bounded on either side by just discernible layers of dark material. Bar = 1.5 µm. (B) Budding cell of *Filobasidium floriforme* (from Moore and Kreger-van Rij 1972). Several generations of budding have produced a ruff around the budding site. Four collars of dark material are evident (arrows) and are considerably thicker than the intervening light layers. Bar = 250 nm.

2.2. Blastic cell division

2.2.1. Bud formation in ascomycetous yeasts: At the start of bud formation in ascomycetous yeasts (see Moore 1987b) vesicles aggregate at the bud site (Moor 1967a, Byers and Goetsch 1975, 1976, Crandall et al. 1977; see Fig. 4: c,l). Some of these vesicles probably contain enzymes (Moor 1967a) that cause plasticizing of the glucans and mannans that, in turn, allow the chitinless cell wall to stretch as the bud balloons out and generate wall layers confluent with those of the parent cell (Fig. 2) (Djaczenko and Cassone 1971). Cytokinesis

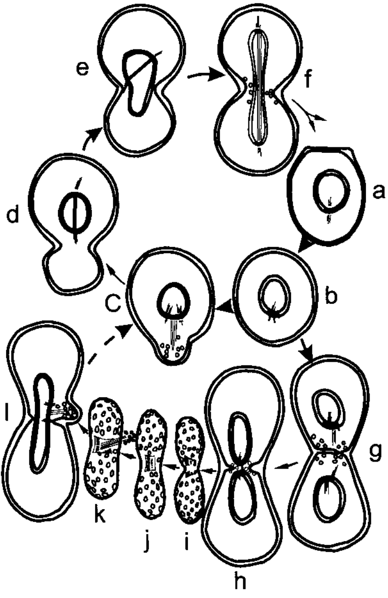


Fig. 4. Cell and nuclear division and conjugation in *Saccharomyces cerevisiae* (redrawn from Byers and Goetsch 1975). (a–f): (a) cell that has reached the critical size necessary for division; (b) division of the spindle pole body (SPB); (c) daughter SPBs start to separate and generate spindle microtubules (note persistence of astral microtubules), the bud initial contains a number of vesicles formed from the endoplasmic reticulum; (d) daughter SPBs migrate to opposite sides of the nucleus, cytokinesis starts; (e) beginning of nuclear division and start of daughter nucleus moving into bud cell; (f) just before cell separation the nucleus becomes dumbbell-shaped, septum formation starts. Conjugation (b, g–l): (b → g) after haploid cells, facilitated by mating substances, become synchronized and gametic, maximum cell-to-cell contact is achieved; (g) lytic vesicles partially dissolve the fusion wall effecting plasmogamy; the SPBs retain their opposing orientation; (h) the SPBs have lead the nuclei to the narrow intercellular opening which will restrict nuclear passage to the extended, SPB-bearing region; (i–k) interpolated perspective views of karyogamy (note the regular size and spacing of nuclear pores). (i) Aster-to-aster fusion and initial contact of the nuclear envelopes; (j) fusion of the two SPBs resulting in the fusion of the contiguous nuclear envelopes; (k) fusion SPB with aster; (l) the first bud of the zygote usually emerges from the fusion site. (l → c) SPB duplication as in a typical cell division.

occurs in the neck of the bud (Fig. 4: f) and involves the formation of a highly ordered ring of 10 nm filaments lying on the interior surface of the plasma membrane and, in glancing electron micrographs of the bud/parent cell isthmus, appear as stria parallel to the long axis (Byers and Goetsch 1976, Byers 1981, Heath et al. 1982, Takeo et al. 1987) (Fig. 2). The initial circumferential space formed by the invaginating plasma membrane is subsequently filled by an electron translucent "constricting ring" of chitin (Cabib and Farkaš 1981). As invagination proceeds, chitin extends across the cell (Bowers et al. 1974) and at completion has a central punctum (Cabib and Bowers 1971). Later, secondary wall layers like those of the homogeneous main cell wall (Cabib 1975) and of similar electron dense appearance are laid down on both sides of the primary septum. Bud separation leaves a flanged discus of chitin on the parent cell as the principal component of the bud scar. In *Candida albicans*, bud scars lack anionic sites (Horisberger and Clerc 1988). The one bud per locus mode of budding limits the number of buds that a parent cell can produce (Mortimer and Johnson 1959, Freifelder 1960, Gyllenberg 1986).

Budding by members of the genera *Hanseniaspora, Nadsonia, Saccharomycodes, Wickerhamia* and their anamorphs is bipolar, i.e., budding occurs just at the poles of the ovoid parent cell. Polar budding can occur repeatedly through the previous bud scar on a broad base, and leaves a succession of appressed, annellidic rings corresponding to the formation of each new bud.

At the time of bud initiation in *Saccharomyces cerevisiae,* the duplicated SPB divides (King and Hyams 1982, Rout and Kilmartin 1990) and the separated SPBs migrate to opposite poles of the nucleus (Fig. 4: a–f). In the course of this movement around the nuclear envelope, the SPBs generate extranuclear mt's (i.e., cytoplasmic or astral) and an intranuclear spindle composed of pole-to-pole and chromosomal mt's. In *Saccharomyces cerevisiae* (Byers and Goetsch 1974, 1975) the SPB acquires a satellite during the latter part of the cdc G_1 phase (prior to bud initiation, see below); early in budding, near the start of the cdc S phase, a pair of SPBs become evident that, by the end of this phase, have formed a complete spindle. Spindles in *Saccharomyces cerevisiae* (Moor 1967b, King and Hyams 1982), *Schizosaccharomyces pombe* (Ding et al. 1993), *Rhodosporidium* (Boekhout and Linnemans 1982), *Taphrina* (Heath et al. 1982), and other yeasts have been shown to be composed of two types of mt's, each composed of 13 proto-filaments (Kilmartin 1981): (1) those mt's of equal length that constitute a prominent central fascicle formed as they pass in opposite directions through the nucleoplasm to become attached, respectively, to the opposite SPB and (2) those mt's of varying lengths that attach to the chromosomes and form opposing May-pole-like splays around the central bundle. In most plant and animal cells the mt attachment site is marked by a primary chromosome constriction, the centromere/kinetochore complex (see Fitzgerald-Hayes 1987); this configuration is absent in *Saccharomyces cerevisiae* (Peterson and Ris 1976). Peterson and Ris (1976) have also shown that the number of SPB-to-chromosome mt's in *Saccharomyces cerevisiae* closely approximates the known number of linkage groups ($n=17$) in haploid and diploid cells. In *Saccharomyces cerevisiae* there are two possible growth ontogenies (Gimeno and Fink 1992): haploid cells express an axial budding pattern in which new buds emerge at the parent/bud cell junction; diploid cells, which are more prevalent in nature, express a polar budding pattern in which new buds emerge from the previous bud cell on the side opposite the junction of that cell with its progenitor, forming a pseudohypha. Towards the end of division, the nucleus becomes dumbbell-shaped with an attenuated membrane isthmus between the incipient sibling nuclei. Telophase spindle elongation takes place across the bud/parent cell isthmus.

2.2.2. Bud formation in basidiomycetous yeasts: Budding in basidiomycetous yeasts occurs repeatedly from a single locus (Fig. 3B). After several generations, this produces a ruff around the budding site composed of accumulated ruptured wall layers (e.g., Baharaeen and Vishniac 1982, Vishniac and Baharaeen 1982, Pore and Sorenson 1990). While the chitin composition of ascomycetous yeasts localized in the bud scar is only 1–2% (Simmons 1989), in *Cryptococcus* spp. it is 5–8% (see Fleet 1985), and in a strain of *Rhodotorula glutinis* it is also relatively high (Berthe et al. 1981). Distribution of chitin (Simmons 1989) can occur evenly throughout the wall (*Cryptococcus neoformans*) or be localized in the collarette (*Malassezia* spp.). In electron micrographs the cell wall appears to have the characteristic basidiomycete profile composed of alternating electron transparent and electron dense layers (Fig. 3B); the dense layers are relatively thick and easy to see, the intervening transparent layers are thin and hard to discern in most micrographs, causing them to appear as transparent space.

The electron transparent layer in Fig. 3A, however, is prominent. In this figure of *Cryptococcus neoformans*, the bud is probably the initial bud cell because the dark material around the exit site is homogeneous. The sandwiched transparent layer, which is about the same thickness as the bounding electron dense layers, is probably chitinous. The transparent layer is the principle component in the bud; further, the wall layers of the bud originate in the parent cell cytoplasm and are not an integral extension of the parent cell wall. Separation probably conveys most of the bud scar to the bud, i.e., the central chitinous transparent layer and the subtending, outer, amorphous electron dense layer (Moore 1989b).

There are several notable differences between nuclear division in ascomycete and basidiomycete yeasts. In ascomycete yeasts (Figs. 1, 4), spindle formation takes place in the parent cell and the nucleus enters the bud directly as it is being formed (Soll et al. 1978). In basidiomycete yeasts (Fig. 5), the initial event of spindle

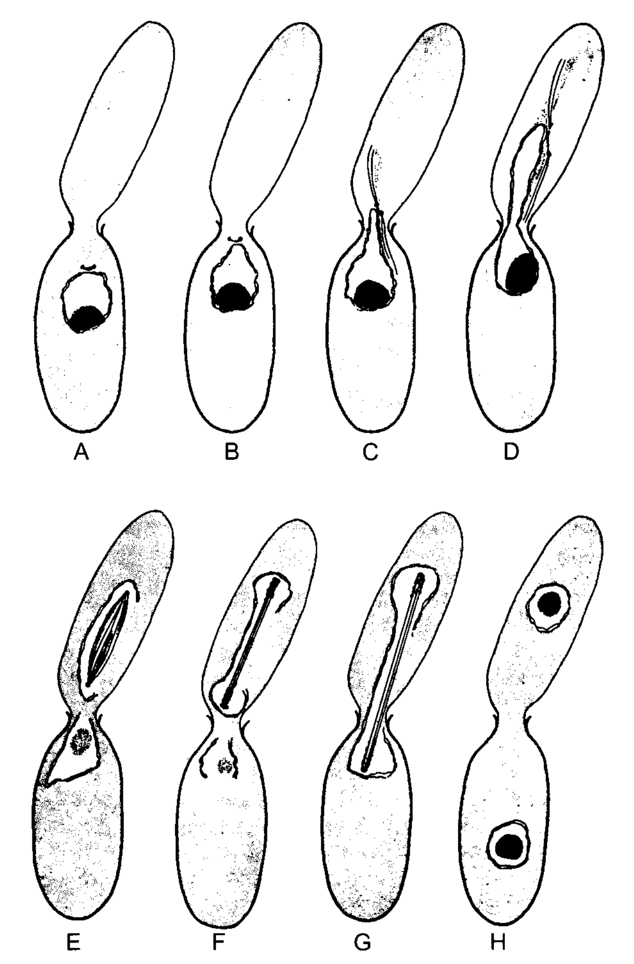


Fig. 5. Nuclear division in *Leucosporidium scottii* (from McCully and Robinow 1972a). (A) Initial bud formation (note the break in the parent cell wall and the orientation of the nucleus and SPB); (B) the nucleus has become pear-shaped with the pointed portion extending towards the bud; (C) nucleus enters the bud led by the asters and SPB; (D) division of SPB, start of daughter SPBs migration; (E) the initial event of elongation takes most of the nucleus into the bud, the small piece left behind containing the nucleolus is absorbed, the nuclear envelope partially dissociates; (F) continued spindle elongation separates the chromosomes; (G) further spindle elongation injects a nascent daughter nucleus into the parent cell; (H) daughter nuclei occupy each end of the budded cell, cytokinesis will occur in the constriction.

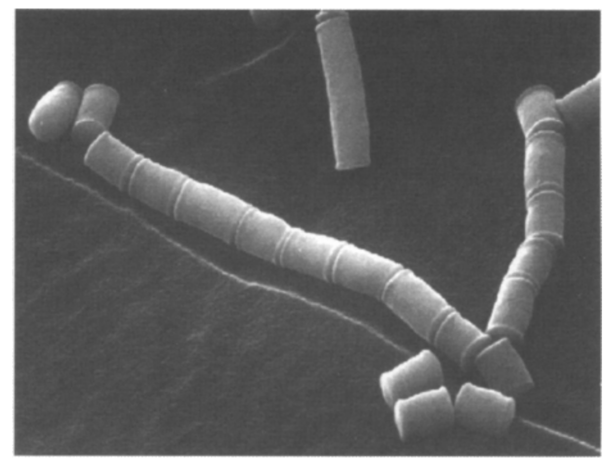


Fig. 6. *Geotrichum candidum*. Scanning electron micrograph showing arthric cell division (from Cole 1975). Length of a single cell is about 13.5 μm.

elongation takes most of the nucleus into the bud, leaving behind a small piece containing the nucleolus, which typically disappears. Division and spindle formation occur within the bud. A second phase of elongation of the intranuclear spindle carries a developing bud nucleus back into the parent cell; during this phase there is also a partial breakdown of the nuclear envelope. This mode of nuclear division has been reported for *Bullera* (Taylor and Wells 1979), *Leucosporidium* (McCully and Robinow 1972a, Boekhout and Linnemans 1982), *Rhodosporidium* (McCully and Robinow 1972b, Abe et al. 1984), and *Ustilago* (Poon and Day 1976a,b).

2.2.3. Conidiogenesis vs. budding: Von Arx (1979b) considered yeast buds as blastoconidia or arthroconidia. There are, however, several fundamental differences between an aerial, hypha-produced conidium (Smith and Anderson 1973) and a single self-replicating yeast cell (Davies 1987): (1) conidiogenous cells draw on, package, and disperse resources of a growing, translocating mycelium (Wessels 1986, McKerracher and Heath 1987, Moore 1987b); (2) hyphal walls and conidia have a sizeable chitin component (Farkaš 1990); ascomycete yeasts, however, lack chitin (see Horisberger and Clerc 1988, Simmons 1989) except for the bud scar, while basidiomycete yeasts have only a small, though perceptible, chitin fraction (Simmons 1989); (3) β-1,4-glucan is a component of conidial walls but not of corresponding hyphal walls (Benhamou et al. 1990); (4) filamentous fungi, unlike yeasts, give inconsistent DBB results (Summerbell 1985) unless treated with cold KOH (Hutchinson and Summerbell 1990).

Conidial differentiation (abstriction) generally requires complete septation (Minter 1984, Cole 1986) and may be arthric or blastic. Arthric abstriction involves progressive septation within a hypha (e.g., *Geotrichum*, Fig. 6) or an existing cell (e.g., *Schizosaccharomyces* and part-spore formation in the Clavicipitales). Blastic abstriction accompanies a brief period of rapid expansion and new wall formation after turgor pressure balloons-out a local softening of the parent cell wall (see Moore 1987b) and ends with septum formation at the base of the new cell. Spore separation (disarticulation) is effected by the breakage or lysis of the hyphal wall opposite the septum.

2.2.4. Pseudohyphae formation: Species of some yeasts have the capacity to form pseudohyphae, a series of coherent, sausage-like, "elongate blastospores" (Scherwitz et al. 1978). Dimorphism in *Candida albicans* is pH regulated. Under conditions of high pH (Anderson and Soll 1986) a constricting filament ring, the anlage of the septum, is initiated a half-hour after the start of bud emergence (Soll 1986). In addition, actin remains clustered in the growing tip rather than becoming redistributed as in normal budding (Adams and Pringle

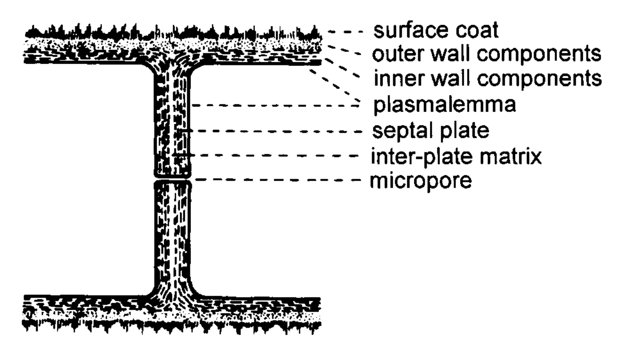


Fig. 7. Model of the crosswall of *Candida albicans* (from Gooday and Gow 1983).

1984, Kilmartin and Adams 1984, Anderson and Soll 1986, Heath 1990). There is also evidence that nutrition can affect which morphology is expressed (Shepherd and Sullivan 1976). Elongate pseudohyphal cells are separated by constricted septa, each with a micropore (Gow et al. 1980) (Fig. 7). When these cells break apart each septum appears composed of two chitinous plates, one going to each cell. In *Saccharomyces cerevisiae* only the diplophase forms pseudohyphae (Gimeno and Fink 1992).

2.2.5. Dimorphism in filamentous species: Dimorphism, in which there is an environmentally controlled, reversible interconversion of yeast and true hyphae or pseudomycelium, Y ↔ M, occurs in a number of species (Romano 1966). The Y-phase, depending on the species, will exhibit characteristic budding of either ascomycetous or basidiomycetous yeasts, and the M-phase, in filamentous species, will be characterized by hyphae with representative ascomycete- or basidiomycete-type septa, and in many species there is a specialized hyphal tip region called a Spitzenkörper (Grove 1978, Howard 1981, Howard and Aist 1979, Hoch and Howard 1980).

For a dimorphic basidiomycete to complete its life cycle, it is necessary that Y-phase cells generate secondary mycelium comprised of hyphae that are either dikaryotic (Flegel 1981) or diploid (Abe and Sasakuma 1986). Hirata et al. (1980) observed that by placing *Tremella mesenterica* Y-phase cells in a hormone solution in which cells of the opposite mating type had been grown, they were able to induce mating-tube formation. The sites and manner of initiation and emergence of these germ tubes was similar to that of buds. As the tube emerged it assumed linear growth and the nucleus, preceded by mitochondria, moved into the tube with the SPB at the leading end of the nucleus.

In *Rhodosporidium toruloides,* mating-tube formation is also under hormonal control (Tsuchiya et al. 1978, Miyakawa et al. 1986). A complete repression of nuclear division occurs with the induction of mating-tube formation; as tube elongation proceeds the nucleus moves into the tube and migrates to the apex. Removal of the hormonal factor causes the resumption of growth by budding (Abe et al. 1977).

Conditions for the phase change are known for a few other species (e.g., Brunton and Gadd 1991, Gimeno and Fink 1992) and there is a growing body of information concerning the molecular biology of the shift between the two morphologies (Schultz et al. 1974, Moore 1987b, Kreisel and Schauer 1989, Maresca and Kobayashi 1989, Kotov and Reshetnikov 1990, Wessels 1990). The yeast/mycelium transition has also been studied electron microscopically (Hirata et al. 1980, Borgia et al. 1989) and computer simulated (Bartnicki-Garcia et al. 1989, Bartnicki-Garcia 1990). There are no substantiated examples of a yeast phase in the Homobasidiomycotina (Laaser et al. 1988, 1989); however, dimorphic life cycles are characteristic of many heterobasidiomycetes (Wells 1994).

2.3. Arthric cell division

2.3.1. *Geotrichum candidum*: In *Geotrichum candidum* (Fig. 6) and other species with similar holoarthric conidiogenesis (Cole and Samson 1979, pp. 108–116) growth is by hyphal tip extension with septation and disarticulation proceeding centripetally. The initial septum is electron transparent but the mature septum has electron dense layers on either side (Steele and Fraser 1973). The end walls of the mature arthrospores are composed only of the respective secondary septal layers and are generally characterized by plasmodesmata or micropores (Table 5).

Karyological differences exist between *Geotrichum candidum* and *Schizosaccharomyces pombe*. Nuclei of *Geotrichum* behave like those of hyphae, dividing and reforming before septogenesis (Cole and Samson 1979, Cole 1986) whereas the nuclei of *Schizosaccharomyces* are typical of yeasts and divide concomitantly with septogenesis (see Hayles and Nurse 1989).

2.3.2. *Schizosaccharomyces pombe*: Mitosis in *Schizosaccharomyces pombe* starts with SPB division and migration of the SPBs to opposite sides of the nucleus (Tanaka and Kanbe 1986). In the course of this movement around the nuclear envelope the SPBs generate extranuclear astral mt's and an intranuclear spindle. Early in division, the central fascicle of continuous SPB-to-SPB mt's assumes a sigmoidal shape. However, reconstructed spindles reveal that mitosis in *S. pombe* is like that of other eukaryotes (Ding et al. 1993). Cytokinesis is not initiated until the nucleus has become dumbbell-shaped. Centripetal invagination of the septum ultimately partitions the sibling nuclei in separate cells. The initial events of cytokinesis occur at the end of karyokinesis (Johnson et al. 1982, Tanaka and Kanbe 1986) before the isthmus connecting the separating sibling nuclei has parted. The electron transparent part of the completed septum fluoresces brightly when stained with Calcofluor or primulin (Streiblová 1981).

Chitin is absent in *S. pombe* (Horisberger and Rouvet-Vauthey 1985): β-glucan and α-galactomannan are interwoven in the cell wall; in the cell plate the former

Table 5
Septal morphology of yeastlike fungi

Morphology	Genus/Species	Reference(s)
Closure-line or macropore	*Arthroascus*	see Moore (1987b)
	Blastobotrys	see Moore (1987b)
	Dipodascus uninucleatus	Kreger-van Rij and Veenhuis (1974)
	Hyphopichia	see Moore (1987b)
	Sporothrix fungorum	see Moore (1987b)
	Stephanoascus	see Moore (1987b)
	Pichia (Hansenula)	see Moore (1987b)
	Yarrowia	see Moore (1987b)
Plasmodesma: random array	*Ascoidea*	see Moore (1987b)
	Dipodascus aggregatus	Kreger-van Rij and Veenhuis (1974)
	Endomyces	see Moore (1987b)
	Geotrichum	see Moore (1987b)
	Galactomyces	see Moore (1987b)
	Guilliermondella	see Moore (1987b)
	Kriegeria	Doublés and McLaughlin (1991, 1992)
	Saccharomycopsis	see Moore (1987b)
	Sporothrix guttuliformis	Smith and Batenburg-van der Vegte (1985)
	Stephanoascus	see Moore (1987b)
	Zygozyma	van der Walt et al. (1991)
Plasmodesma: circular array	*Dipodascus magnusii*	see Moore (1987b)
	Dipodascus ovetensis	see Moore (1987b)
	Dipodascus tetrasperma	see Moore (1987b)
Dolipore/Parenthesome: O_1/P_2	*Cerinosterus*	see Moore (1987b)
$O_2/P_{3,4}$	*Bulleromyces*	Boekhout et al. (1991a)
	Filobasidium capsuligenum	see Moore (1987b)
	Hyalodendron	see Moore (1987b)
	Moniliella	see Moore (1987b)
	Sirobasidium	Moore (1979)
	Rhynchogastrema	Metzler et al. (1989)
	Tetragoniomyces	Oberwinkler and Bandoni (1981)
	Tremella	see Moore (1987b), Oberwinkler (1985), Berbee and Wells (1988)
	Trichosporon asahii	Guého et al. (1992b)
	Trichosporon coremiiforme	Guého et al. (1992b)
	Trichosporon cutaneum	Guého et al. (1992b)
	Trichosporon laibachii	Guého et al. (1992b)
	Trichosporon moniliforme	Guého et al. (1992b)
	Trichosporonoides	see Moore (1987b)
	Trimorphomyces	Oberwinkler and Bandoni (1983)
O_2/P_5	*Wallemia*	see Moore (1986, 1987b)
O_2/P_6	*Filobasidium floriforme*	see Moore (1985)
	Filobasidiella neoformans	see Moore (1985)
	Filobasidiella depauperatus	see Moore (1985)
	Itersonilia	Boekhout (1991b)
	Phragmoxenidium	Oberwinkler et al. (1990c)
	Stilbotulasnella	see Moore (1985)
	Syzygospora	Ginns (1986)
	= *Carcinomyces*	Oberwinkler and Bandoni (1982a)
	= *Christiansenia*	Oberwinkler and Bandoni (1982a)
	Trichosporon mucoides	Guého et al. (1992b)
	Trichosporon sporotrichoides	Guého et al. (1992b)

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Table 5, *continued*

Morphology	Genus/Species	Reference(s)
Nanopore	*Atractiella*	Oberwinkler and Bandoni (1982c)
	Agaricostilbum	Oberwinkler and Bandoni (1982c)
	Bensingtonia	Boekhout et al. (1992b)
	Camptobasidium	Marvanová and Suberkropp (1990)
	Chionosphaera	Oberwinkler and Bandoni (1982c)
	Erythrobasidium	Suh et al. (1993a)
	Exobasidium	Khan et al. (1981b), Patrignani et al. (1989)
	Graphium	Oberwinkler et al. (1982)
	Helicobasidium	Bourett and McLaughlin (1986)
	Herpobasidium	Oberwinkler and Bandoni (1984)
	Kondoa	Suh et al. (1993a)
	Leucosporidium fellii	Suh et al. (1993a)
	Leucosporidium scottii	Moore (1972)
	Naohidea	Oberwinkler (1990)
	Occultifer	Oberwinkler (1990)
	Pachnocybe	Oberwinkler and Bandoni (1982c), Kleven and McLaughlin (1989)
	Phleogena	Oberwinkler and Bandoni (1982c)
	Platygloea	Aoki et al. (1986)
	Rhodosporidium dacryoideum	Suh et al. (1993a)
	Rhodosporidium sphaerocarpum	Moore (1972)
	Rhodosporidium toruloides	Johnson-Reid and Moore (1972)
	Sporidiobolus johnsonii	Moore (1972)
	Sporidiobolus	Boekhout et al. (1992b)
	Ustacystis	Bauer et al. (1995)
Septobasidium-like	*Atractogloea*	see Moore (1987b), Oberwinkler and Bandoni (1982b)
	Septobasidium	Dykstra (1974)
SLN (slightly lipped nanopore)	*Cystofilobasidium capitatum*	Oberwinkler et al. (1983)
	Cystofilobasidium lari-marini	Suh and Sugiyama (1993a)
	Mrakia frigida	Suh et al. (1993a)
	Tilletia caries	Moore (1972), Roberson and Luttrell (1987a,b)
	Tilletia foetida	Moore (1972)
	Tilletia indica	Roberson and Luttrell (1987a,b)
	Tilletiopsis fulvescens	Boekhout et al. (1992b)
	Tilletiopsis minor	Boekhout et al. (1992b)
	Ustilago avenae	Bauer et al. (1989)
	Ustilago maydis	Moore (1972), O'Donnell and McLaughlin (1984b)
Amorphous	*Cintractia sorghi-vulgaris*	Moore (1972)
	Cystofilobasidium infirmo-miniatum	Marvanová and Suberkropp (1990)
	Entyloma	Boekhout et al. (1992b), Moore (1972)
	Spacelotheca	Bauer et al. (1989)
	Sympodiomycopsis	Suh et al. (1993a)
	Tilletia barclayana	Moore (1972)
	Tilletiaria	Boekhout et al. (1992b)
	Tilletiopsis pallescens	Boekhout et al. (1992b)
	Ustilago nuda	Moore (1972)
	Ustilago tritici	Bauer et al. (1989)

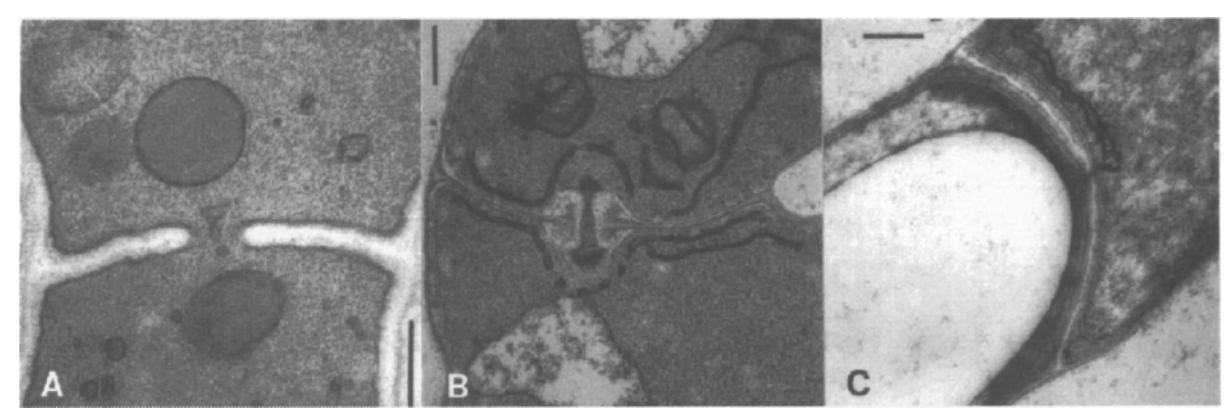


Fig. 8. Perforate septa of various fungi (from Moore 1972). (A) Ascomycete type of an unidentified anamorph. Bar = 250 nm. (B) Basidiomycete type *(Polyporus biennis)*. Bar = 250 nm. (C) Basidiomycete type (*Leucosporidium scottii*). Bar = 250 nm.

is associated with the primary septum while the latter is associated with the secondary septum; on the cell surface, β-glucan, in exact opposition to α-galactomannan, is detected only on the wall layers generated by division but not on those growing by extension. As a consequence of the lysis of the primary septum, the new end wall of each cell is one of the secondary wall layers formed on either side of it. Each new end wall (see Miyata et al. 1990) is like the original wall layer of the parent cell but thinner; one sibling cell, however, retains a jacket of old wall material. As a result of this mode of cell division, the pattern of wall scars is different for each cell (Streiblová 1981). When growth resumes, it starts at the old ends (see Hagan and Hyams 1988, Mitchison 1990).

Ultrastructural observations of *S. pombe* are complemented by light microscopic and biochemical studies. According to Hagan and Hyams (1988): (1) cytoplasmic mt arrangement most closely resembles that of filamentous fungi (possibly a consequence of cylindrical cell shape); (2) interphase occupies ~75% of the cdc during which time cells more or less double in size (from 7 to 14 μm); (3) certain cytoplasmic mt's, which longitudinally span the cell and disappear at the time of mitosis, may play a role in positioning the nucleus and in establishing the cell's polarity (this array is reestablished by a pair of MTOCs at the cell equator after the mt's of mitosis depolymerize); (4) disruption of mt function leads to a controlled loss of wall deposition, resulting in distended or branched cells (in *Saccharomyces cerevisiae,* however, enlargement continues normally in the absence of mt's even though nuclear positioning becomes random); (5) F-actin undergoes structural rearrangements during the cdc at three distinct points – (a) in G_2 phase, at the transition from monopolar to bipolar cell growth; (b) in M phase, at the initiation of septation; and (c) at the cell division/G_1 phase boundary at the reinitiation of monopolar growth. Cellular distribution of F-actin varies during the course of the cdc (Marks et al. 1986, Steer 1990): (1) during final cell elongation, prior to division, it is predominantly located at the growing ends of the cell; (2) in the course of division, the least amount is observed during karyokinesis, while during cytokinesis it becomes concentrated equatorially; (3) as division proceeds to cell separation, it becomes concentrated at both the new (post-division) ends, as well as, again, at the original ends.

γ-Tubulin has been found to occur throughout the cell cycle and to be associated with mt development in the regions of the SPBs (Horio et al. 1991). It probably has a role in mt formation because mutants in which its function is disrupted display condensed, undivided chromosomes with aberrant spindle structures that result in cell death.

3. Hyphae

3.1. Septal ontogeny

Cell division in filamentous species is first discernible when a narrow annulus of the plasma membrane is formed (Girbardt 1979). Centripetal growth of this annulus partitions the cytoplasm with an infolded membrane. New wall material is deposited on the outer surface of the membrane: beneath the lateral wall layer and along the ingrowing annulus (Moore 1965). Septal ontogeny can have any of several different conclusions depending on the taxon or the particular cell type: (1) in most species of the Chytridiomycota and Zygomycota and in some yeasts, particularly the Schizosaccharomycetales (Eriksson et al. 1993, Kurtzman 1993a), constituting a small percentage of fungi, the final ingrowth of the annulus results in complete cytokinesis and the formation of an abscissional septum; (2) in a few species of yeastlike fungi the center of the mature septum is characterized by a punctum or minute micropore; (3) in the great majority of fungi – certain mucors and trichomycetes (see Moore 1987b, Saikawa 1989), ascomycetes, and basidiomycetes – the mature septum is perforate (Fig. 8) (Markham 1994). These studies have demonstrated that septal ultrastructure is a robust indicator of taxonomic relationships (Moore 1985, 1987b, 1988b, 1989a, 1994, 1996a).

3.2. Ascomycete-type

3.2.1. Perforate septa: In ascomycete hyphae, septa are, for the most part, homogeneous and electron transparent.

Pores (Fig. 8A) are large (of sufficient size for the passage of nuclei), have rounded rims, and frequently are accompanied on either side by Woronin bodies (Markham and Collinge 1987), which are membrane bound electron dense inclusions with paracrystalline material. Hyphae of *Ambrosiozyma* (synonym *Hormoascus*) have greatly centrally-thickened septa (van der Walt and von Arx 1985).

3.2.2. Micropores and plasmodesmata: In some yeasts and yeastlike fungi a minuscule membrane cylinder in the center of the final septum (Fig. 7) may appear as either a micropore (with a central channel) or a closure line (that looks like a punctum in surface view) (Table 5). Micropores are not the same as plasmodesmata. Distribution of plasmodesmata can be random or circular (Table 5). General reviews of plasmodesmata can be found in Marchant (1976) and Robards and Lucas (1990). Van der Walt et al. (1991) consider the plasmodesmatal canals in *Zygozyma* to be further evidence for the connection between the Lipomycetaceae and Dipodascaceae. Multiperforate septa in the basidiomycete *Kriegeria eriophori* (Platygloeales) (Kao 1956, Doublés and McLaughlin 1991) may have an ontogeny similar to those in ascomycetes.

3.3. Basidiomycete-type

3.3.1. Introduction: Basidiomycete walls and septa, in toto, are tripartite (Figs. 3, 8B,C). They are composed of a central electron transparent layer, probably chitin or chitinous, bounded on either side by electron dense layers. The basidiomycetous yeast *Malassezia* is unusual in having corrugated inner walls (Simmons and Ahearn 1987). Septa, with rare exception, are perforate. Clamp connections are a common feature of dikaryotic hyphae (secondary and tertiary mycelium). Basidiocarps are composed of tertiary mycelium.

3.3.2. Septa: The basidiomycetes, from the evidence of morphology and molecular biology, are a natural group (Bruns et al. 1992, Suh and Sugiyama 1994, Swann and Taylor 1995b). Septa in several orders are characterized by the dolipore/parenthesome (d/p) complex (Moore 1985, 1996a). Hoch and Howard (1981) have shown that the toroidal swellings of the dolipore septum are an artifact of conventional chemical fixation. Such barrel-shaped swellings are absent in samples that have been quick-frozen and freeze substituted. Nevertheless, these fixation artifacts appear to be reproducible and therefore interpretable within a systematic context (Table 5). Dolipore septa possess three basic structures (Figs. 8B, 9): (1) a barrel-shaped dolipore, (2) occlusions within the pore channel, and (3) parenthesomes on either side that delimit the pore domain. The diameter of the pore channel is 89 to 266 nm (Patton and Marchant 1978) and is occupied by opposing cylindroidal occlusions. In many taxa, the outer portion of the occlusion is homogeneous and electron dense (O_1 type: Figs. 8B, 9); in

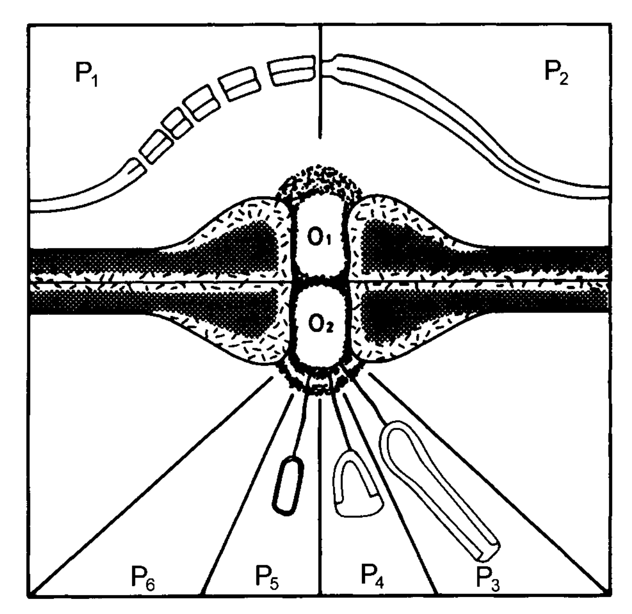


Fig. 9. Summary diagram of the basic variations to be found in dolipore/parenthesome (d/p) septa of the phylum Basidiomycota (from Moore 1985). Particular taxa are generally associated with certain d/p combinations (Moore 1996a): O_1/P_1 septa occur only in the Hymenomycetes and Gasteromycetes; O_1/P_2 septa characterize the Clavariomycetes and the monomorphic jelly fungi (Auriculariales and Exidiales) (Wells 1994); $O_2/P_{3,4,6}$ septa occur only in the orders Tremellales and Filobasidiales (Table 5); O_2/P_5 septa are unique to *Wallemia sebi* (see Moore 1986).

the Tremellales s. str. and Filobasidiales (Moore 1996a) it appears as a striated band (O_2 type: Fig. 9). Moore (1985) postulated that the pore results from the final ingrowth of the annulus being physically blocked. The dolipore domain is delimited by parenthesomes (Fig. 9), double membrane septal caps with an intervening line that are differentiated from the endoplasmic reticulum. Depending on the taxonomic group, parenthesomes may be perforate (P_1: Fig. 8B), imperforate (P_2), vesiculate (P_3–P_5), or absent (P_6). The several morphologies of the d/p septal complex are diagnostic of specific major taxa (Table 5; Fig. 9).

4. Sexual cycles

4.1. Class Hemiascomycetes

4.1.1. Ascospore formation: Ascospore formation starts with meiosis which, like mitosis, is intranuclear (Moens and Rapport 1971, Peterson et al. 1972, Moens 1974). Ascospore development in the Euascomycetes (Marchant 1979, Mims et al. 1990, van Wyk and Wingfield 1991) and in *Schizosaccharomyces octosporus* (Czymmek and Hammill 1989) occurs by free cell formation. A similar ontogeny occurs in most budding yeasts, but with some significant differences. An ascal sac is not formed and after meiosis the sibling nuclei do not separate but persist as lobes attached to a central mass of nucleoplasm; each lobe has an SPB that forms internal spindle mt's (Fig. 10). Each SPB becomes associated with a forespore envelope (FSE), a double membrane cupule that comes to enclose its respective lobe. An FSE

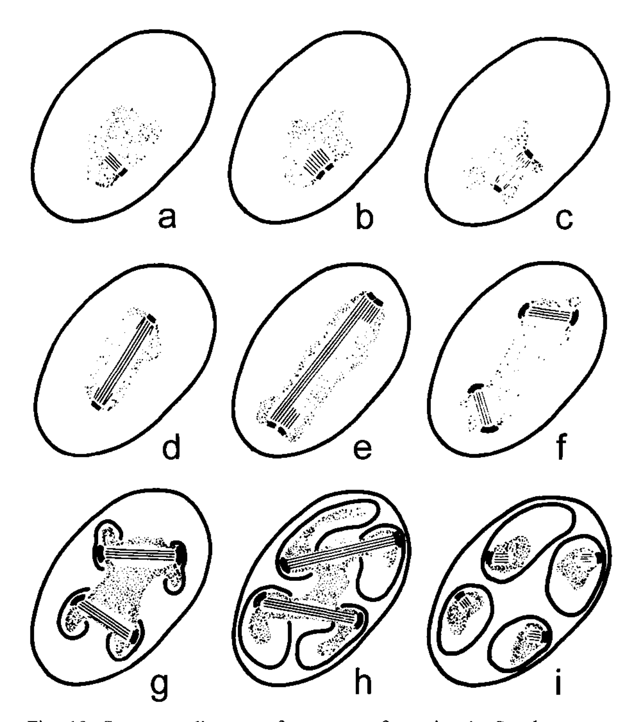


Fig. 10. Summary diagram of ascospore formation in *Saccharomyces cerevisiae* (from Moens and Rapport 1971). At the start of sporulation (a, b) the SPB replicates and the daughter SPBs move apart (c, d) and generate the primary spindle; (e) the primary SPBs, in turn, replicate when the meiosis I spindle has achieved its maximum length; (f) the resulting pairs of secondary SPBs separate to form a pair of shorter meiosis II spindles. Between stages (f) and (g) two significant events occur: 1) the formation, outside each primary SPB plaque, of secondary SPB plaques along with vesicles that are the anlagen of the forespore envelopes (FSE); 2) the formation of a nuclear bulge forming adjacent to each SPB that enlarges while the central part of the nucleus shrinks. (g) Around each nuclear lobe an FSE forms and increases at the margins (h), following the contours of the nuclear lobes (Guth et al. 1972, Beckett et al. 1973, Moens 1974). (i) The pursing of the FSEs finally isolates the lobes, initiating the ascospores. The excluded central portion of nucleoplasm becomes part of the epiplasm. The primary ascospore wall is deposited in the FSE as the nuclear lobe is being severed and it may become quite thick while there is still a gap in the spore wall.

initially appears as a flattened vesicle just outside the SPB with a lumen of the same density as the rest of the cytoplasm; it soon becomes compressed and filled with a dense matrix (Moens 1971, 1974, Zickler and Olson 1975). The primary ascospore wall is deposited in the FSE as the nuclear lobe is being severed and it may become quite thick. A comparable ontogeny occurs in *Wickerhamia fluorescens* (Rooney and Moens 1973a,b) and *Eremothecium ashbyi* (Rosing 1987).

The primary ascospore wall layer is electron transparent, smooth, and generally is of uniform thickness. Secondary wall material is deposited on the outside of the primary wall under the outer membrane and is usually electron dense and distinctively patterned (Kurtzman et al. 1972, 1980b, Kreger-van Rij and Veenhuis 1975a, 1976a, Kreger-van Rij 1977a, 1979, Kurtzman and Kreger-van Rij 1976, Mikata and Banno 1987). Sometimes, however, the secondary wall material has nearly the same density as the primary wall, e.g., *Ascoidea* (Ashton and Moens 1979), *Cephaloascus* (Ashton and Moens 1979), *Pichia* (Bandoni et al. 1967, Black and Gorman 1971), *Hanseniaspora* (Kreger-van Rij 1977b), and *Saccharomycodes* (Simmons and Ahearn 1985). In *Sporopachydermia* the secondary wall, uniquely, becomes very thick and irregular (Rodrigues de Miranda 1978, Kreger-van Rij 1978, Simmons and Ahearn 1987).

In *Debaryomyces hansenii* karyogamy generally occurs in the budding isthmus after which only one nuclear lobe, FSE, and subsequent ascospore is formed, while the rest of the nucleus disappears (Kreger-van Rij and Veenhuis 1975a). Nuclear separation prior to FSE formation and primary wall deposition occurs in *Ascoidea* (Ashton and Moens 1979), *Cephaloascus* (Ashton and Moens 1979), *Dipodascopsis* (Curry 1985), *Pichia* (Black and Gorman 1971), *Saccharomycopsis* (Kreger-van Rij and Veenhuis 1975b, Ashton and Moens 1979), *Schizosaccharomyces* (Yoo et al. 1973, 1990), and *Taphrina* (Syrop and Beckett 1972).

4.1.2. Conjugation: When suspensions of the mating types of *Pichia canadensis* (synonym *Hansenula wingei*) are mixed, up to 80% of the cells fuse in pairs (Conti and Brock 1965). The process of agglutination causes considerable wall deformation as maximum cell-to-cell contact is achieved. Subsequently, the cells elongate in the contact region, the walls fuse and then partially dissolve, and plasmogamy occurs. Nuclei move toward the isthmus and quickly fuse on contact. Following karyogamy a bud develops from the conjugation tube; its occupation by the zygote nucleus establishes the teleomorph phase (see Crandall et al. 1977).

A similar ontogeny occurs in *Saccharomyces cerevisiae* (Osumi et al. 1974, Byers and Goetsch 1975, Cross 1988) (Fig. 4: g–l). Conjugation, here and probably in other heterothallic species, requires synchronization of haploid cycles that are regulated by mating substances. SPBs of gametic cells have satellites and extranuclear mt's that, initially, are associated with the developing intercellular connection and, later, with the SPB of each nucleus as they migrate together. SPBs retain their opposing orientation as they lead the nuclei towards the intercellular gap where they meet and fuse. The narrow intercellular opening restricts nuclear passage to the extended, SPB-bearing region. Karyogamy proceeds by the fusion of the two SPBs and contiguous nuclear envelopes. Formation of the fusion SPB marks the end of the prolonged cdc G_1 arrest, coincident with conjugation, and is followed by SPB duplication as in a typical cell division. The first bud of the diplophase usually emerges from the fusion site. In *Saccharomycopsis (Guilliermondella) selenospora* (Kreger-van Rij and Veenhuis 1976a), conjugation tubes from different hyphae fuse at their tips and karyogamy occurs in the channel; subsequently a new wall is formed in the channel and the parent cells become asci.

4.2. Phylum Basidiomycota

Species that produce highly diverse basidiocarps are made up of tertiary mycelia, specialized hyphae that have the characteristic dolipore/parenthesome septal complex. Meiosis occurs in basidia that may, after division, remain entire (holobasidia) or become variously septate (phragmobasidia). Generally four ballistosporic basidiospores are produced on basidia but in some taxa (i.e., Gasteromycetes, Filobasidiales) spores are passively released.

Members of the Uredinales are highly evolved plant parasites and have complex life cycles with as many as five spore stages. Teliospore germination produces a metabasidium which, after meiosis, becomes a four-celled phragmobasidium. Dikaryotic mycelium of the rusts lacks clamp connections, is generally intercellular, and is limited to parts of leaves and other aerial organs of the host; basidiocarps are not formed. Sorus and spore ontogeny *are* determinant and regular (Hiratsuka and Sato 1982).

Teliospores in several orders are formed from dikaryotic hyphae; initially binucleate, they become diploid by maturity. During germination the diploid nucleus may divide mitotically before it or a sibling nucleus moves into a metabasidium and undergoes meiosis. Ontogeny of thin-walled teliospores in *Rhodosporidium toruloides* (Johnson-Reid and Moore 1972, Abe et al. 1977) is interpreted as follows: clamp-like hookcells emerge near septa as outgrowths on a broad base and develop into crozier-like, double clamp configurations in which the penultimate cells, after further development, become

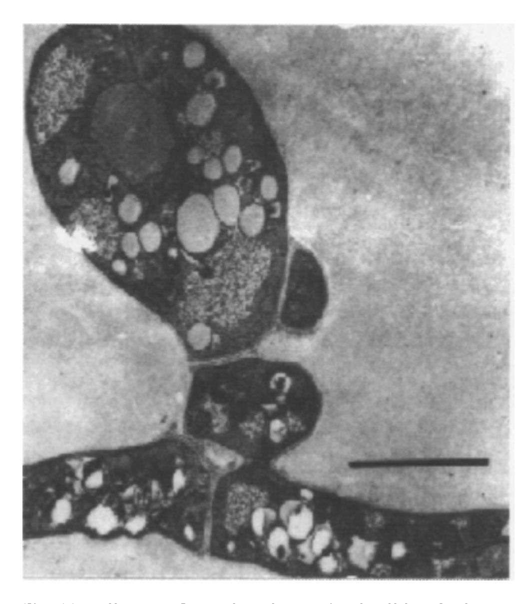


Fig. 11. Teliospore formation above a hookcell in *Rhodosporidium* (from Johnson-Reid and Moore 1972). The spore forms from a penultimate cell in a "crozier" over "clamp" system in which the hookcell ("clamp") is the antipenultimate cell that later fuses with the ultimate cell (see Fig. 12D/E). Bar = 2.5 µm.

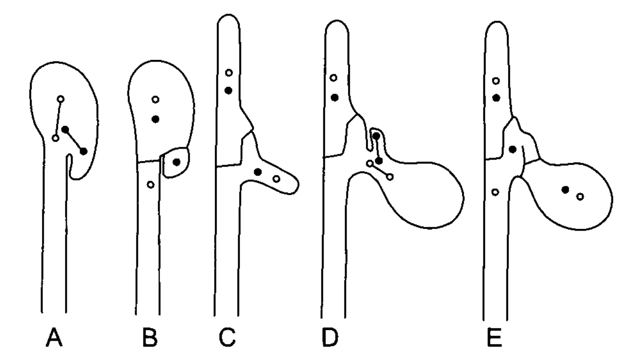


Fig. 12. Interpretation of teliospore formation in *Rhodosporidium,* original drawing based on Seyfert (1927) and Johnson-Reid and Moore (1972): (A–B) terminal hookcell type that mimics crozier formation (see Banno 1967); (C–E) lateral double-clamp or hookcell-("crozier")-on-clamp type.

teliospores (Fig. 11); teliospores may also be formed from apical crozier-like cells. A diagrammatic interpretation of teliospore development is given in Fig. 12. Similar teliospore ontogenies appear to be present in *Leucosporidium* (Moore 1972), *Rhodosporidium (Kondoa) malvinellum* (Fell 1970b, Yamada et al. 1989a), and *Sporidiobolus pararoseus* (de Hoog and Boekhout 1982).

Teliospore germination usually starts with a mitotic division in which one nucleus remains in the spore while the other enters the developing basidium (Zundel 1953); iterations of this ontogeny probably explains the formation of additional basidia (Fischer and Holton 1957, Christensen 1963, Durán and Safeeula 1968, Ingold 1983). Exceptions have been reported in *Tilletia*: Goates and Hoffman (1987) found that meiosis in several species occurred within the teliospores before the metabasidium emerged. Therrien et al. (1988) also found that teliospores of *Tilletia indica* were multinucleate at the time of germination. The order Ustilaginales (smuts) is characterized by metabasidia that become septate after meiosis. The basal cell of the phragmobasidium occupies the teliospore; the basidiospores (also called sporidia) generated from each cell usually arise near a septum. Meiosis in *Ustilago maydis* (O'Donnell and McLaughlin 1984a, O'Donnell 1992) is similar to that reported for other basidiomycetes (Uredinales excepted). In *Exobasidium* (Blanz 1978, Mims et al. 1987), basidial initials develop within the dikaryotic stroma and give rise to lateral, uninucleate holobasidia on the lower leaf surface. After meiosis four to seven sterigmata develop per basidium. The tip of each sterigma enlarges into a basidiospore which remains attached abaxially; a hilar appendix body and discharge droplet are not produced. Instead, basidiospores are passively released and grow in a yeastlike manner (Mims and Richardson 1987, Mims et al. 1987).

4.3. Phylum Ustomycota

Moore (1972, 1987b, 1988a, 1996a–d, 1997) proposed

that there are basic phylogenetic differences between the rusts and the smut fungi and assigned the latter group to the phylum Ustomycota. Although the phylum Ustomycota has not been adopted in this book, readers may become familiar with this alternative system of classification by consulting the foregoing references.

Chapter 6

Chemotaxonomy based on the polysaccharide composition of cell walls and capsules

H.J. Phaff

Contents

1. Introduction

Comparison of the composition of polysaccharides that make up the cell wall and capsule of capsulated yeasts has had a significant impact on the systematics and phylogeny of yeasts. Such information has proven to be of value mainly at the generic and suprageneric level. Only a small number of species of yeasts has been investigated in depth with regard to the constituent monosaccharides and derivatives, and even fewer studies have focused on the structural details of their constituent polysaccharides. The cell wall composition of additional species is often inferred from information obtained with related species.

Historical reviews on the development of our knowledge of the yeast cell envelope have been published by Phaff (1971, 1977), Fleet and Phaff (1981), Fleet (1984, 1985, 1991), Golubev (1991a), Cabib (1981), and Cabib et al. (1984). An excellent review on the assembly of the various cell wall components of yeasts was recently published by Klis (1994). Most information based on chemical analyses has been derived from studies of cell walls from *Saccharomyces cerevisiae* and a few related ascomycetous species. It is often assumed that other multilaterally budding ascomycetous yeasts have a similar composition, although there is evidence from studies with cell wall lytic enzymes that there are quantitative as well as structural differences among the component polysaccharides of different species (Tanaka et al. 1966).

2. Ascomycetous cell envelopes

Ascomycetous budding yeasts contain an alkali-insoluble acid-insoluble β-(1 → 3)-glucan as the major structural component. Associated with this polysaccharide is a low proportion of an alkali-insoluble acid-soluble highly branched β-(1 → 6)-glucan and an alkali-soluble β-(1 → 3)-glucan with a significant number of β-(1 → 6)-bonds in its branched structure (see Fleet and Phaff 1981) for structural details). There is a very low chitin content in ascomycetous, multilaterally budding yeasts, which is mainly confined to the bud scar areas of the wall and thus the proportion of chitin varies with the number of daughter cells that have been produced (Cabib 1981).

The remaining major component of the cell wall of these yeasts is a glucomannan-protein complex (Ballou 1976). More recently, Van Rinsum et al. (1991) and Montijn et al. (1994) have explored the attachment of the glucomannoproteins to the other polysaccharides of the cell wall. Their findings show that the glucomannoproteins contain β-(1 → 6)-linked glucose-containing side chains (in addition to the already known N- and O-linked chains) to the protein moiety of the mannoprotein. Evidence presented suggests that the glucose-containing side chains might be extended with β-(1 → 3)-linked glucose chains that could interweave with the β-(1 → 3)-glucan fibrils in the wall and thus anchor the glucomannoproteins. Presumably this linkage is eliminated during the extraction procedures of the glucomannoprotein.

Significant differences in the carbohydrate moiety have been demonstrated, particularly in the mannan side chains of the branched molecule (Ballou 1976, 1982). Selective acetolysis has played a major role in the elucidation of yeast mannan structure (Kocourek and Ballou 1969, Ballou 1976). With controlled acetolysis, the α-(1 → 6) linkages of the mannan backbone are selectively cleaved, and the oligosaccharides thus formed (representing the mannose side chains including one mannose residue originally part of the backbone) can be separated according to size by gel filtration. The elution pattern appears to be reproducibly characteristic of a particular yeast species, thus providing a "fingerprint" of this mannan.

The molar ratios of the various oligosaccharides can be determined from the areas under the elution peaks. A comparison of elution patterns (fingerprints) of a significant number of species has shown that this approach can constitute a useful parameter in yeast taxonomy. Although this method provides information on the proportion and size of the oligosaccharide side chains in various mannans, it does not reveal without further analysis the types and distribution of the linkages between the mannose units in the side chains.

Another approach to the fingerprinting of mannans was developed by Gorin and Spencer (for a comprehensive review see Gorin and Spencer 1970). They determined and compared proton magnetic resonance spectra of

isolated mannans from a large number of yeast species. These spectra provided sensitive criteria for distinguishing mannans of different species or for showing their similarity. At 70°C the H-1 and DOH signals give complex patterns in the τ 4.0–5.0 region. The chemical shifts of the H-1 proton signals depend on the structure of the parent anhydrohexose unit, the position(s) substituted in the unit, the structure of the substituent unit, the structure of the aglycone unit if one is present, and the position(s) substituted in such an aglycone unit. With this technique, a large number of water-soluble mannose-containing polysaccharides could be distinguished. Gorin et al. (1969b) concluded that polysaccharides with similar proton magnetic resonance spectra have related chemical structures, and thus mannans from different yeast species that produce similar or identical spectra indicate that these species are closely related.

This technique has been helpful in confirming or rejecting postulated anamorphs and teleomorphs of the same species (Manachini 1979), and in establishing tentative groups of seemingly related species in heterogeneous asexual genera such as *Candida* (Spencer and Gorin 1969a). Proton magnetic resonance spectra have also been used (a) to evaluate various types of evidence for proposed phylogenetic lines in *Pichia* (synonym *Hansenula*) species (Spencer and Gorin 1969b), (b) to obtain presumptive evidence for the occurrence of β linkages in the side chains of mannans, resulting in distinctive H-1 signals at a higher field than τ 4.55 (Gorin et al. 1969a), and (c) to detect sugars other than mannose in the mannans (Gorin and Spencer 1968).

As mentioned briefly above, multilaterally budding ascomycetous yeasts contain about 1–2% chitin that is confined almost entirely to the bud scar areas. However, a small percentage (around 0.1%) is located throughout the lateral walls (Molano et al. 1980, Roncero et al. 1988a,b). Most of the evidence is based on studies with *S. cerevisiae* and it is assumed that other budding ascomycetous yeast species have a similar chitin distribution. In contrast, budding yeasts of basidiomycetous affinity, for example in species of the basidiomycetous anamorphs *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* (Bartnicki-Garcia 1968), have a much higher chitin content (up to approximately 10%) and this form of chitin is present as microfibrils and not concentrated as granular chitin in bud scar zones. The chitin content of walls of filamentous ascomycetes is significantly higher than in *S. cerevisiae*, e.g., in species of *Saccharomycopsis* and the euascomycete *Eremascus*. The chitin content of hyphal forms of *Candida albicans* has been found much higher than that in the budding form of that species. The chitin content of species of the bipolarly budding genus *Nadsonia* is much higher than that of species of the genera *Hanseniaspora* and *Saccharomycodes*, suggesting that *Nadsonia* is not closely related to the last two genera (reviewed by Phaff 1971, Fleet 1991). Ribosomal RNA comparisons also suggest *Nadsonia* to be only distantly related to the other two genera (Kurtzman and Robnett 1994a). It should be added that detailed knowledge about the chitin content of species of most yeast genera is still lacking or incomplete, making its significance to yeast systematics uncertain.

The cell wall composition of the fission yeast *Schizosaccharomyces* differs in several major respects from that of the budding ascomycetous yeasts. Earlier conclusions that species of this genus do not contain chitin have proven to be incorrect. Sietsma and Wessels (1990) reported a small proportion (0.5% of the dry weight of the wall) of a partially acetylated glucosaminoglycan, that is covalently linked to β-(1 → 3)-glucan of the wall. Treatment of the walls with chitinase greatly decreased the content of alkali-insoluble β-(1 → 3)-glucan and increased the fraction of alkali-soluble β-(1 → 3)-glucan, suggesting that the chitinous fraction of this yeast is bound to the alkali-insoluble β-(1 → 3)-glucan component. These authors also demonstrated the presence of a chitin synthase in a membrane fraction of *S. pombe*. Further characterization of the chitin synthase gene of *S. pombe* has been reported by Bowen et al. (1992).

Species of *Schizosaccharomyces* contain in addition to the β-glucans of the budding yeasts another major structural polysaccharide, i.e., α-(1 → 3)-glucan, which is soluble in alkali (Bacon et al. 1968, Meyer and Phaff 1980). The mannan component of *Schizosaccharomyces* is also different from that of the budding yeasts in that it has single galactose units attached to the backbone rather than oligomannose chains (Ballou 1976). The fission yeasts thus have a unique cell wall composition and do not appear closely related to any other group of ascomycetous yeasts, an observation consistent with comparisons of rRNA (Kurtzman and Robnett 1994a). It should be pointed out that the occurrence of α-(1 → 3)-glucan in *Schizosaccharomyces* is not unique, because it is also found as a major cell wall component in *Cryptococcus* and *Phaffia* (Bacon et al. 1968, Meyer and Phaff 1977) and in numerous species of filamentous fungi (Fleet and Phaff 1981), but this polysaccharide appears lacking in *Rhodotorula* species (Meyer and Phaff 1977).

The presence of unusual sugars or sugar derivatives in the cell wall has had a large impact on the systematics of yeasts at the generic or suprageneric level. As mentioned above, the genus *Schizosaccharomyces* is characterized by the occurrence of *galactomannan* in the wall. Weijman (1977) compared the cell wall composition of *Dipodascus* and *Dipodascopsis* and found that the monotypic genus *Dipodascopsis* contains glucuronic acid in the cell wall but this uronic acid is absent in *Dipodascus* spp. This finding supports the separation of *Dipodascopsis* from *Dipodascus*.

3. Basidiomycetous cell envelopes

The presence or absence of D-xylose in the cell envelope or whole cell extracts also has had an important bearing on yeast systematics at the generic level. Von Arx and Weijman (1979) placed yeast species of heterobasidiomycetous

affinity (based on mode of bud formation) in two families depending on the presence or absence of D-xylose. The Sporobolomycetaceae (lacking xylose and frequently containing fucose) included species of *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, and some species of *Candida*. The Filobasidiaceae (containing xylose and usually glucuronic acid) included species of *Bullera*, *Cryptococcus*, *Filobasidium*, *Phaffia* and some species of *Candida* and *Trichosporon*. Later, Weijman and Rodrigues de Miranda (1983) reported xylose in the majority of *Bullera* species, but a few *Sporobolomyces* species also contained xylose. No definitive steps for reclassification of the nonconforming species were taken by these authors. Subsequently, Suzuki and Nakase (1988a) established by a more sensitive analytical technique that xylose was lacking in *Bullera salicina* and *Bullera tsugae*, and Nakase and Itoh (1988) transferred these two species to the genus *Sporobolomyces*. Nakase and Suzuki (1986c) confirmed the finding by Weijman and Rodrigues de Miranda that *Sporobolomyces puniceus* contains xylose in the cells and they transferred this species to the genus *Bullera* as *B. punicea* comb. nov. These new combinations emphasize the lesser importance of ballistospore morphology and other minor morphological details in the systematics of these genera as compared to chemotaxonomic and other molecular criteria such as Co-Q (Nakase et al. 1991a). Studies on the presence of unusual sugars and sugar derivatives in cell walls have also given support for the separation of species in the genera *Candida* and *Trichosporon* with ascomycetous and basidiomycetous affinity (von Arx and Weijman 1979, Weijman and Rodrigues de Miranda 1988). Sugiyama et al. (1985), in their study of the genera *Rhodosporidium*, *Leucosporidium*, *Cystofilobasidium*, *Filobasidium*, and *Filobasidiella*, emphasized the importance of the presence of xylose in dividing the species into two major groups.

An important criterion of relating the carotenoid-containing new genus *Saitoella* to the ascomycetes, was the absence of xylose in the cells (Goto et al. 1987a). In spite of the many useful results referred to above, it should be emphasized that the carbohydrate composition of the yeast cell envelope is not a decisive criterion by itself in yeast systematics, but is valuable only in combination with other chemotaxonomic and molecular approaches, including ribosomal DNA sequence analysis. Such a polyphasic approach has been advocated by a number of authors for a reliable taxonomic system.

4. Capsular polysaccharides

The composition of capsular polysaccharides (Golubev 1991a) has also been found useful as an aid in yeast systematics. Early studies on the slimy phosphomannans found in certain species of *Pichia* (*Hansenula* spp.) pointed to their common ancestry (Wickerham and Burton 1962). The presence of a linear mannan with alternating β-(1 → 3)- and β-(1 → 4)-bonds in some *Rhodotorula* species and an acidic heteropolysaccharide containing xylose, mannose and glucuronic acid in *Cryptococcus*, has supported the transfer of several species of *Rhodotorula* to *Cryptococcus* (Phaff and Spencer 1969) and has established a relationship between *Cryptococcus* and the heterobasidiomycetous genus *Tremella* (Slodki et al. 1966). It has been suggested by Slodki and Wickerham (1966) that the lack of D-galactose in the capsule of *Lipomyces lipofer* would be a useful criterion to separate this species from *L. starkeyi*, because these species are difficult to separate on the basis of the usual assimilatory reactions. However, as pointed out by Phaff and Kurtzman (1984), mutations have been reported where a capsulated strain of *L. lipofer* changed into a microcapsulated variant with a much higher galactose content (Gorin et al. 1978). The same authors also found that an occasional strain of *L. tetrasporus* contained an unusually low level of galactose in its capsule, making the differentiation between *L. tetrasporus* and *L. lipofer* difficult on this basis. Another complication in the use of chemical composition of capsular material in yeast systematics is the problem of purity of the preparation to be analyzed (Golubev 1991a). Because of the difficulty of separating capsular polysaccharides from exocellular, excreted polysaccharides and fragments of cell walls, there appears to be doubt that at least some of the literature reports were based on sufficiently purified capsular preparations.

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Chapter 7

Electrophoretic comparisons of enzymes

M. Yamazaki, C.P. Kurtzman and J. Sugiyama

Contents

1. Introduction

The differences in amino acid sequences found among enzymes of different organisms is a reflection of organismal genetic divergence. Amino acid substitutions can often be detected from the extent of migration shown by enzymes on electrophoretic gels and the visualized patterns are termed zymograms. Electrophoretic comparisons of enzymes have been used to study taxonomic relationships among molds and mushrooms (Blaich and Esser 1975, Jones and Noble 1982, Micales et al. 1986, Nasuno 1971, Nealson and Garber 1967, Okunishi et al. 1979, Royse and May 1982, Schmidt et al. 1977, Stout and Shaw 1973, 1974, Sugiyama and Yamatoya 1990, Toyomasu and Zennyozi 1981, Yamatoya et al. 1990, Zambino and Harrington 1992, Zamir and Chet 1985) as well as among yeasts and yeastlike fungi (Baptist and Kurtzman 1976, Sidenberg and Lachance 1983, 1986, Smith et al. 1990b, Yamazaki and Goto 1985, Yamazaki and Komagata 1981, 1982a,b). The focus of our review is to examine the extent of genetic resolution provided by electrophoretic comparisons of enzymes and the use of this technique for solution of taxonomic problems among yeasts.

2. Commonly used methods

Different electrophoretic techniques, including starch gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), and isoelectric focusing, can be used. Although gel support media differ, PAGE generally gives good separation and repeatability for yeasts. Advantages of the different methods of electrophoresis have been discussed by Murphy et al. (1990). The method used will often be determined by availability of equipment and expertise.

3. Preparation of cell-free extracts for electrophoresis

Cells can be disrupted with a mechanical cell homogenizer (e.g., B. Braun, Melsungen, Germany) using a coolant such as liquid CO_2. After treatment, insoluble debris and unbroken cells are removed by centrifugation, and the liquid supernatant is used directly for electrophoretic analyses of enzymes (Sidenberg and Lachance 1983, 1986, Smith et al. 1990b, Yamazaki and Komagata 1981). The electrophoretic apparatus often used is for vertical slab gels. Polyacrylamide gels have been used by Davis (1964), Sidenberg and Lachance (1983, 1986) and Yamazaki and Komagata (1981), but starch gels also give good results (Baptist and Kurtzman 1976, Nealson and Garber 1967, Royse and May 1982, Singh and Kunkee 1977, Zamir and Chet 1985). Further details may be found in the work of Bonde et al. (1993) and Micales et al. (1986).

4. Enzymes commonly compared

The staining procedures for detection of enzymes in electrophoretic gels have been described by Siciliano and Shaw (1976). Yamazaki and Goto (1985), Yamazaki and Komagata (1981, 1982a,b, 1983a,b), and Yamazaki et al. (1982, 1983, 1985) compared the following enzymes: fructose-1, 6-bisphosphate aldolase (EC 4.1.2.13), hexokinase (EC 2.7.1.1), phosphoglucomutase (EC 2.7.5.1), alcohol dehydrogenase (EC 1.1.1.1), lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37), 6-phosphogluconate dehydrogenase (EC 1.1.1.41), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase (EC 1.4.1.4), fumarase (EC 4.2.1.2), esterase (EC 3.1.1.1), catalase (EC 1.11.1.6) and tetrazolium oxidase. These enzymes were chosen because they play important roles in the metabolism of yeasts. The enzymes superoxide dismutase (EC 1.15.1.1), alkaline phosphatase (EC 3.1.3.1), α-glucosidase (EC 3.2.1.20), β-glucosidase (EC 3.2.1.21), and exo-β-glucanase (EC 3.2.1.58) were examined by Sidenberg and Lachance (1983, 1986).

5. Data analysis

The application of electrophoretic data in systematic studies has been discussed in detail by Buth (1984) and Murphy et al. (1990). After staining, gels are dried under a vacuum with warming, and the relative mobilities of the enzyme bands are calculated as the ratio of the distance that the enzymes move from the origin to the distance that the tracking dye moves (Yamazaki and Komagata 1981).

Similarity values for the electrophoretic patterns of the enzymes are calculated by the formula

$$\%S = NS/(NS + ND) \times 100,$$

with S = similarity value; NS = number of enzymes showing identical mobilities; ND = number of enzymes showing different mobilities.

Similarity for each enzyme is calculated by the following formula:

$$\%S = 2\,NAB/(NA + NB) \times 100,$$

with S = similarity value; NAB = the number of enzyme bands with identical relative mobilities; NA = the number of enzyme bands of strain A; NB = the number of enzyme bands of strain B (e.g., Hamamoto et al. 1986a). Clustering is achieved by means of the unweighted-average linkage technique by the method of Sneath and Sokal (1973).

Sidenberg and Lachance (1983, 1986) used reciprocal averaging to ordinate the strains as a function of correlated electromorphs. The amount of information provided by each enzyme is evaluated by the following measure of entropy (I_j):

$$I_j = (n \times t_j \times \ln t_j) - \sum_{i=1}^{n} \left[(a_{ij} \times \ln a_{ij}) + (t_j - a_{ij}) \times \ln(t_j - a_{ij}) \right],$$

where n is the number of strains, t_j is the total number of different electromorphs for the jth enzyme, and a_{ij} is the number of electromorphs of that enzyme present in the ith strain. The results are expressed in a matrix having the dimensions $n \times p$, where n strains are described by the presence (scored as 1) or absence (scored as 0) of each of p electromorphs. Reciprocal averaging is used to ordinate rows and columns of a frequency matrix and simultaneously reveals correspondences between two kinds of information (i.e., strains and electromorphs).

6. Resolution of taxa

In this review, selected groups of taxa are compared with respect to their isozyme profiles and their extent of nucleic acid divergence. By this approach, perspective is added to the resolution offered from use of the different techniques.

6.1. Ascomycetous yeasts

6.1.1. *Debaryomyces*: Yamazaki and Komagata (1982a) examined the electrophoretic patterns of 10 enzymes of *Torulopsis candida* (= *Candida famata*), a species then regarded as the anamorph of both *Debaryomyces hansenii* and *D. marama*, and found that strains of *T. candida* fell into three groups. Nakase and Suzuki (1985a,b) compared phenotypically similar strains of *D. hansenii* and related yeast taxa with respect to DNA base composition, nuclear DNA (nDNA) reassociation, ubiquinone structure, PMR spectra of mannans, and serological reactions. The 50 strains of *D. hansenii* examined were classified into three groups (I, II, III) based on nDNA reassociation (Price et al. 1978). Group I consisted of two subgroups, Ia and Ib, which are considered to represent two distinct varieties. The first and second groups of Yamazaki and Komagata (1982a) corresponded to subgroup Ia and group III, respectively. These groups and subgroups could be discriminated from one another by maximum growth temperature, assimilation of propylene glycol, and electrophoretic patterns of glucose-6-phosphate dehydrogenase and malate dehydrogenase. *Debaryomyces coudertii* and *D. marama*, which resemble *D. hansenii*, were distinguished from each other and from strains of groups I, II and III by electrophoretic patterns of the preceding two enzymes. On the basis of this information, groups I, II and III, and subgroups Ia and Ib were taxonomically described by Nakase and Suzuki (1985b).

Kurtzman and Robnett (1991) and Yamada et al. (1991a) estimated evolutionary affinities among species of *Debaryomyces* from partial rRNA (18S and 26S) gene sequences. Their phylogenetic studies showed that *D. hansenii, D. coudertii, D. marama,* and *D. nepalensis* are closely related. These results are congruent with data from other chemotaxonomic comparisons, including zymograms and nDNA relatedness, which suggest the four taxa to be biologically separate members of a closely related species complex.

6.1.2. *Kluyveromyces*: Species of the genus *Kluyveromyces* have been compared with respect to enzyme patterns and nuclear DNA relatedness. The type strains of 20 phenotypically defined species of *Kluyveromyces* were examined by gel electrophoresis of 11 isofunctional enzymes (Sidenberg and Lachance 1983). Polymorphisms were evident in most of the enzymes studied. Each type strain had a unique pattern when all enzymes were considered. The results of a multivariate analysis of the electrophoretic patterns supported the division of the genus into 13 species. Enzyme electrophoresis provided evidence that widespread gene flow does not occur between yeasts which are able to hybridize in the laboratory. Additionally, Sidenberg and Lachance (1986) described the results of an electrophoretic characterization of seven enzymes to assess the degree of variability within the *Kluyveromyces marxianus* complex and to determine the taxonomic relationships of these populations based on reproductive isolation as it appears to have occurred in nature. Some strains of *Candida* considered to be anamorphs of *Kluyveromyces* as well as one strain each of *C. sake, Saccharomyces cerevisiae* and *Pichia fluxuum,* were also examined. The enzyme patterns readily substantiated the relationships between putative anamorphs and teleomorphs and the nonmember status of strains not belonging to the genus. A multivariate analysis of the enzyme patterns indicated that isolates belonging to

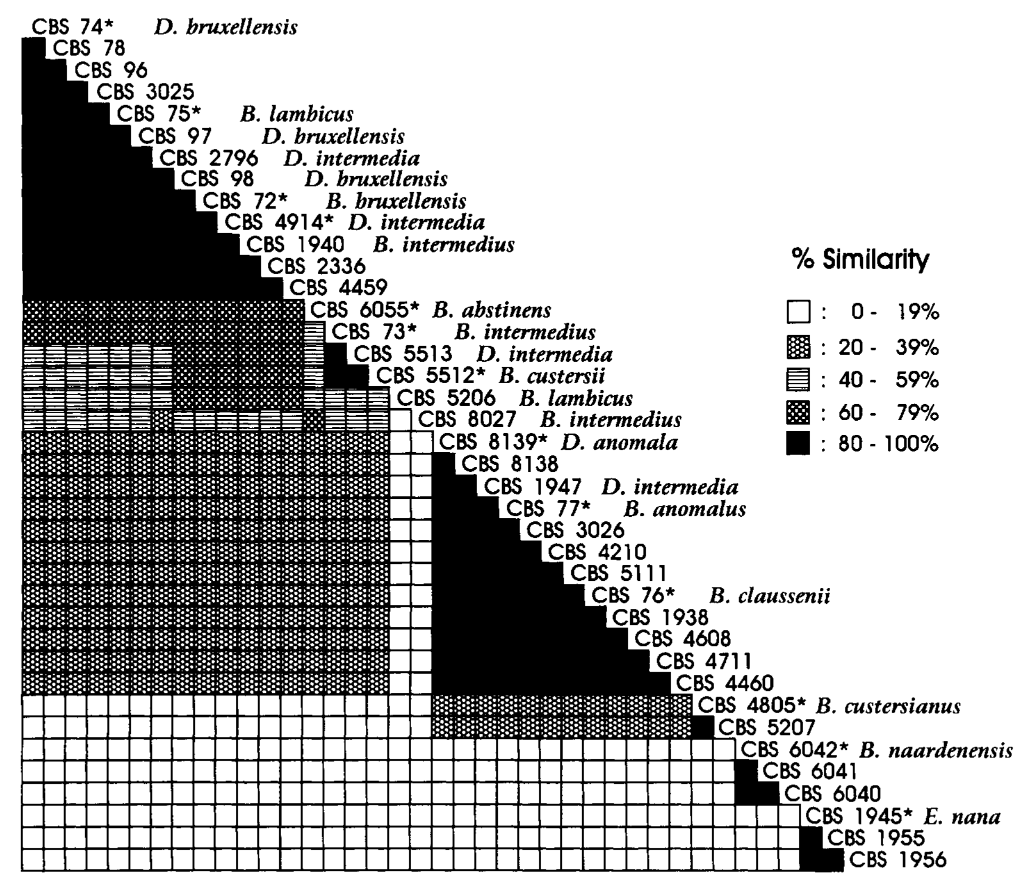


Fig. 13. Similarity of strains of *Brettanomyces, Dekkera* and *Eeniella* based on relative electrophoretic mobilities of five cellular enzymes (Smith et al. 1990b).

K. lactis and *K. marxianus* DNA reassociation groups are not phenotypically continuous with one another. Results from studies of nuclear DNA reassociation demonstrated the same species groups within *Kluyveromyces* as defined from the preceding electrophoretic comparisons (Fuson et al. 1987, Vaughan-Martini and Martini 1987b).

6.1.3. *Brettanomyces/Dekkera/Eeniella*: Smith et al. (1990b) examined the taxonomic status of various species of the teleomorphic genus *Dekkera* and the anamorphic genera *Brettanomyces* and *Eeniella* by electrophoretic comparison of five enzymes (Fig. 13), with respect to nDNA relatedness (Table 6), and with respect to physiological reactions. Enzyme patterns demonstrated the presence of two *Dekkera* species [*D. anomala* with anamorph *B. anomalus* (=*B. claussenii*), *D. bruxellensis* (=*D. intermedia*) with anamorph *B. bruxellensis*] and three *Brettanomyces* species [*B. bruxellensis* (=*B. abstinens, B. custersii, B. intermedius, B. lambicus*), *B. custersianus* and *B. naardenensis*] that were recognized from low (0–29%) similarity values. Strains of each species (except *D. bruxellensis*) showed similarity values of 80–100%. *D. bruxellensis* strains were divided into two clusters: one cluster has a high similarity value (85–100%) while strains of the second cluster showed a similarity value of 38–70%. Using the guideline that strains with a similarity level of 40% and greater belong to the same species (Hamamoto et al. 1986a), the two clusters were considered conspecific. *Eeniella nana* showed a unique enzymic pattern that differed from other *Brettanomyces* and *Dekkera* species (0–5% similarity). Results from nDNA reassociation supported the conclusions drawn from electrophoresis of enzymes because strains within each taxon showed 70% or greater complementarity.

More recently, Boekhout et al. (1994) determined partial nucleotide sequences (ca. 600 nucleotides) of a divergent domain near the 5′ end of the 26S rDNA gene for species of *Dekkera* (anamorph *Brettanomyces*), *Hanseniaspora* (anamorph *Kloeckera*) and *Eeniella* to reconstruct the phylogenetic relationships of these taxa. As shown in Fig. 14, monophyly of the *Dekkera/Brettanomyces/Eeniella* clade is strongly supported by a high bootstrap value (100%), as is the branch supporting *D. anomala* and *D. bruxellensis*. However, the branching order for *B. naardenensis, E. nana* and *B. custersianus* is only weakly resolved (51%). Taxonomic conclusions based on comparisons of enzyme patterns (Fig. 13), nDNA relatedness (Smith et al. 1990b, Table 6) and partial 26S rDNA sequence divergence (Fig. 14) are generally complementary.

Table 6
Extent of nDNA reassociation among strains of *Brettanomyces* and *Dekkera*

Species	Strain no.	Percent DNA relatedness		
		D. anomala CBS 8139[a]	*D. bruxellensis* CBS 74[a]	*D. intermedia* CBS 4914[a]
Dekkera anomala	CBS 8139[a]	–	18	7
Brettanomyces anomalus	CBS 77[a]	100	–	–
	CBS 7250	100	–	–
D. bruxellensis	CBS 74[a]	18	–	100
B. bruxellensis	CBS 72[a]	–	100	–
D. intermedia	CBS 4914[a]	7	100	–
	CBS 1947	99	–	24
B. intermedius	CBS 73[a]	–	–	100
B. claussenii	CBS 76[a]	100	–	–
	CBS 4068	100	–	–
B. custersianus	CBS 4805[a]	23	24	–
B. custersii	CBS 5512[a]	–	–	88
B. lambicus	CBS 75[a]	–	98	–
	CBS 5206	–	–	99
B. naardenensis	CBS 6042[a]	16	–	16

[a] Type strain.

6.2. Basidiomycetous yeasts

6.2.1. *Rhodosporidium/Rhodotorula/Erythrobasidium/Cystofilobasidium*: Yamazaki and Komagata (1981) used slab gel electrophoresis with specific staining for seven enzymes to compare 108 strains belonging to the genera *Rhodotorula* and *Rhodosporidium*. Subsequently, their data were analyzed numerically (Hamamoto et al. 1986a). Strains with coenzyme Q-10 were divided into six clusters, strains with coenzyme Q-9 separated into two clusters, and strains with coenzyme Q-8 were assigned to two clusters. At ca. 60% or greater similarity, strains of the teleomorphic species *Rhodosporidium diobovatum, R. sphaerocarpum, R. dacryoidum, R. malvinellum,* and *R. infirmominiatum* segregated into species-level clusters. Among these species, the extent of intraspecific nDNA relatedness (*R. malvinellum* was not compared) was high (64–99%). Consequently, nDNA complementarity, type of ubiquinones, and similarity of enzyme patterns (see Hamamoto et al. 1987) argue that the preceding taxa represent well-defined species.

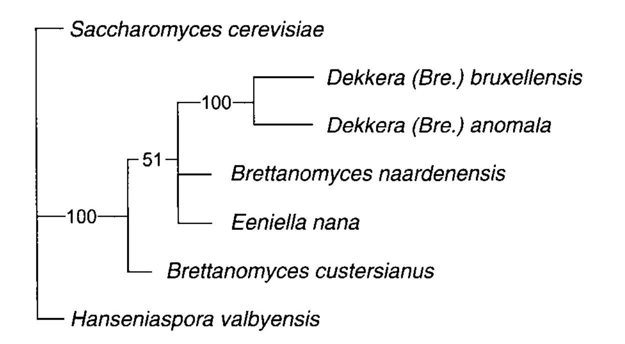


Fig. 14. Phylogenetic tree showing relationships among species of *Brettanomyces*, *Dekkera* and *Eeniella*. The tree was calculated using the branch-and-bound option of the program PAUP 3.1.1 and based on ca. 600 nucleotides from the 5′ end of 26S rDNA (recalculated from the data of Boekhout et al. 1994). Numbers indicate bootstrap values.

Divergent enzyme patterns have served to signal phylogenetic diversity among other red yeasts. For example, three strains of *R. lactosa* divided into two groups based on their ubiquinone systems (Yamada and Kondo 1973), enzyme patterns (Yamazaki and Komagata 1981) and DNA base composition (Hamamoto et al. 1986b). The new species *Rhodotorula hasegawae* was proposed by Yamada and Komagata (1983) for one strain of *R. lactosa* having coenzyme Q-10(H2). Subsequently, Hamamoto et al. (1988a) found that the species produced a holobasidium from a single cell without prior mating, and described it in the new teleomorphic genus *Erythrobasidium* which was tentatively placed in the Filobasidiaceae. Recently, Sugiyama and Suh (1993), Suh and Sugiyama (1994) and Suh et al. (1993a) have suggested that *E. hasegawianum* occupies an unusual position within the basidiomycete lineage having simple septa as defined by Swann and Taylor (1995b). In another example, the anamorphic species *Rhodotorula sinensis* showed ca. 60% similarity to *Rhodosporidium infirmominiatum*. Extent of relatedness between this taxon and species of *Cystofilobasidium*, as determined from similarity of isozymes, is shown in Fig. 15 (Hamamoto et al. 1986a,b, 1988b). The relationships depicted are strongly supported by partial rRNA sequence comparisons (Yamada and Kawasaki 1989a, Fell et al. 1992).

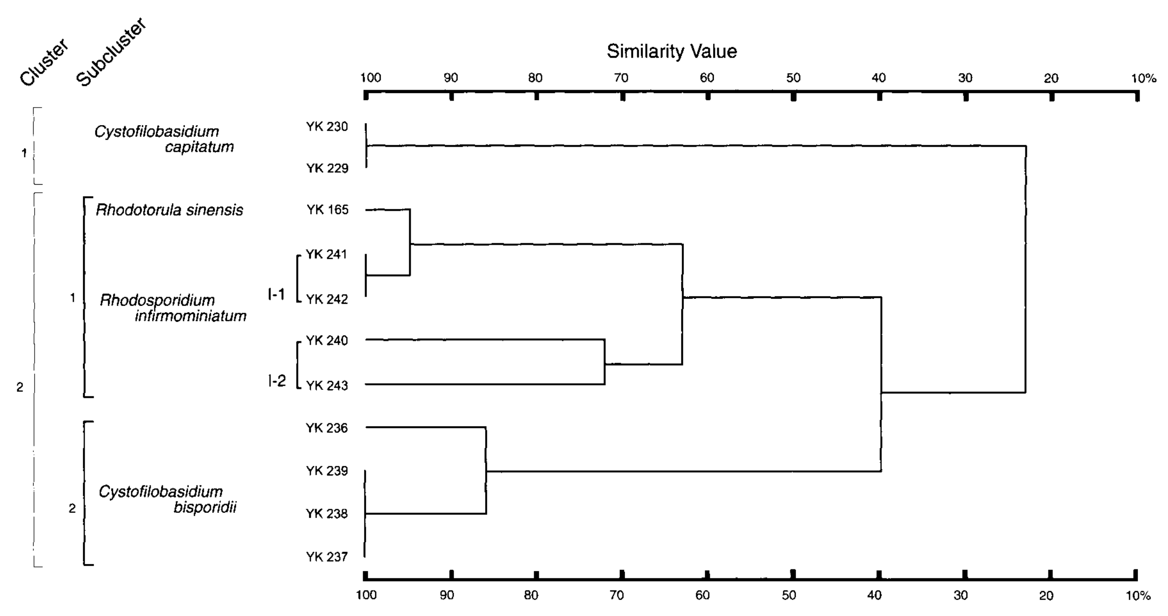


Fig. 15. Dendrogram calculated from similarity values of the electrophoretic mobilities of enzymes from species in the genera *Rhodosporidium*, *Cystofilobasidium* and *Rhodotorula* having coenzyme Q-8 (Hamamoto et al. 1986a).

6.2.2. The *Sterigmatomyces* complex: Yamada and Banno (1984a) and Yamada et al. (1988a,b) reassigned species classified in *Sterigmatomyces* to four anamorphic genera having the following characteristics:

- *Sterigmatomyces*: separation of buds at mid-sterigma, coenzyme Q-9, cellular xylose absent.
- *Tsuchiyaea*: separation of buds at mid-sterigma, coenzyme Q-9, cellular xylose present.
- *Kurtzmanomyces*: distal separation of buds from the sterigma, coenzyme Q-10, cellular xylose absent.
- *Fellomyces*: distal separation of buds from the sterigma, coenzyme Q-10, cellular xylose present.

It is unclear from phenotypic data whether species assigned to the preceding genera and the teleomorphic genus *Sterigmatosporidium* are biologically distinct. Yamada et al. (1986a,b) examined genealogical relationships among these genera using numerical analysis based on the similarities of electrophoretic patterns of seven enzymes. All strains of *Sterigmatomyces elviae, S. halophilus,* and *Sterigmatosporidium polymorphum* compared gave a uniform enzyme pattern within their respective species. Strains of *S. halophilus* showed 43% similarity with strains of *S. indicus*. The similarity between *S. elviae* and *S. halophilus* was only 14%. Kurtzman (1990a) addressed the question of species separation among this group of taxa from the perspective of nDNA relatedness. Consistent with the preceding electrophoretic comparison of *Sterigmatomyces,* only *S. halophilus* and *S. indicus* showed more than low levels of nDNA relatedness. *S. halophilus* and *S. indicus* had nearly 100% nDNA relatedness and are considered to be conspecific although their similarity based on enzyme patterns is comparatively low (43%).

The three species of *Fellomyces (F. fuzhouensis, F. penicillatus, F. polyborus),* and *Kurtzmanomyces nectairei,* formerly classified in *Fellomyces,* had quite different enzyme patterns (0% similarity). Furthermore, the similarity between *K. nectairei* and *Sterigmatosporidium polymorphum* was only 14%. The extent of nDNA relatedness among *Fellomyces* species ranged from 0–8%, indicating that all of these taxa are genetically separate species. Similar low levels of nDNA relatedness were detected between *Fellomyces* species, *K. nectairei,* and *S. polymorphum,* a species whose anamorph is typical of the genus *Fellomyces*. Comparisons of enzyme patterns and nDNA relatedness showed that *Sterigmatomyces fuzhouensis* and *S. tursiopsis* are conspecific.

Guého et al. (1990) examined relationships among species in the preceding genera with respect to sequence comparisons of selected regions of small (18S) and large (26S) subunit rRNAs. Their sequence data support the concept of Yamada and Banno (1984a) and Yamada et al. (1988a,b) that *Sterigmatomyces, Fellomyces, Tsuchiyaea* and *Kurtzmanomyces* represent separate genera. The molecular data also indicate that the genus *Fellomyces* is closely related to the teleomorphic genus *Sterigmatosporidium.* The only point of doubt concerns *Tsuchiyaea wingfieldii* which invariably clusters closely with *Filobasidiella neoformans* in rRNA analyses.

In conclusion, the extent of genetic resolution derived from electrophoretic comparisons of selected enzymes generally parallels results from nDNA reassociation. Both methods resolve to the sibling species level, whereas rRNA sequence comparisons detect relationships at greater genetic distances.

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Chapter 8

Mycocins (Killer Toxins)

W.I. Golubev

Contents

1. Introduction

Almost 30 years ago strains of *Saccharomyces cerevisiae* were found to secrete a protein (killer toxin) lethal to other strains (sensitive) of the same species (Makower and Bevan 1963). These strains (killers) were immune to their own toxins. Some strains (neutral) neither secreted the toxin nor were they sensitive to it. Subsequent studies revealed that in most killer strains of *S. cerevisiae,* the toxin is produced by a linear dsRNA. Another dsRNA encodes a protein that encapsulates the dsRNAs into a virus-like particle (VLP). The VLPs are noninfectious and are transmitted by vegetative cell division or through sexual fusion (Tipper and Bostian 1984, Schmitt and Tipper 1990).

The killer phenomenon provides an excellent model system to study host–virus interactions in eukaryotic cells (Wickner 1989), and to investigate the mechanisms of protein processing and secretion (Douglas et al. 1988). Possible uses for killer phenomena, which have aroused great interest, include the protection of industrial fermentations against contaminating yeasts (Rosini 1989) and the differentiation of pathogenic (Morace et al. 1983/84) and patented strains (Vaughan-Martini et al. 1988). In addition, killer dsRNA was shown to have interferon-inducing activity (Nosik et al. 1984) and the killer toxin has a therapeutic effect (Polonelli et al. 1986).

For a proper understanding of the killer phenomenon, it is necessary to emphasize that it is not unique for yeasts. Production of proteins having toxicity specific for related organisms, which is associated with specific immunity, is known for smut fungi (Koltin 1988), paramecia (Quackenbush 1988), slime molds (Mizutani et al. 1990) and bacteria (Konisky 1982). The bacterial protein antibiotics are termed bacteriocins and, to accent the general nature of such antagonistic interactions, it is preferable to call yeast killer toxins mycocins and killer strains mycocinogenic strains.

In the yeasts studied, mycocin determinants are cytoplasmically inherited. Killer systems similar to that of *Saccharomyces cerevisiae* dsRNA exist in *Hanseniaspora uvarum* (Zorg et al. 1988), *Sporidiobolus johnsonii (S. salmonicolor)* (Kitaite and Čitavičus 1988), and *Cystofilobasidium bisporidii* (Karamysheva et al. 1991). Mycocin production in *Kluyveromyces lactis* (Stark et al. 1990), *Pichia acaciae* (Worsham and Bolen 1990) and *P. inositovora* (Ligon et al. 1989) is associated with linear dsDNA plasmids. The killer phenotypes in *Candida glabrata* (Sriprakash and Batum 1984) and some strains of *S. cerevisiae* (Goto et al. 1990) are encoded by chromosomal genes. In many cases the genetic basis for killer activity has not been characterized but is presumed to be of chromosomal origin.

2. Characteristics of mycocins

All yeast mycocins studied are proteinaceous. They are either proteins or glycoproteins that consist of two or three subunits. In most yeasts, the molecular weights of secreted toxins are around 10 000–20 000 D (Bussey and Skipper 1975, Middelbeek et al. 1979, Pfeiffer and Radler 1982, 1984, Ashida et al. 1983, Ohta et al. 1984, Yamamoto et al. 1986, Yokomori et al. 1988, Suzuki and Nikkuni 1989) although the molecular weights of glycoprotein mycocins produced by *Kluyveromyces lactis* and *Pichia anomala* are much higher (100 000 D and greater) (Sugisaki et al. 1983, Stark and Boyd 1986, Kagiyama et al. 1988, Sawant et al. 1989). The secreted K1 mycocin (20 658 D) of *Saccharomyces cerevisiae* consists of α and β disulfide-linked polypeptides with a high content of hydrophobic and charged amino acids (Palfree and Bussey 1979, Zhu et al. 1987). The K1 precursor, which has been synthesized in cells, has a large single-strand polypeptide in which the α and β subunits are separated by an interstitial glycosylated γ-region (Bostian et al. 1984, Lolle and Bussey 1986).

After exposure to most mycocins, growing sensitive cells exhibit a reduction in intracellular pH and leakage of potassium ions, ATP and other cellular intermediates. Amino-acid transport and proton pumping to the culture medium are also inhibited (Skipper and Bussey 1977, Middelbeek et al. 1980b–d, Ashida et al. 1983). All of these effects are indicative of an increase in proton permeability in mycocin sensitive cells (de la Pena et al. 1981). Apparently, a mycocin becomes incorporated into the cytoplasmic membrane and creates ion permeable channels (Kagan 1983, Martinac et al. 1990). The overall

effect of pore formation disrupts the cell electrochemical potential across the membrane and eventually results in cell death. Many, but not all yeast mycocins are pore-forming glycoproteins. The *Kluyveromyces lactis* mycocin, for example, does not elicit leakage of potassium ions and ATP but causes an arrest in the G1 phase of the cell cycle (White et al. 1989, Butler et al. 1991). The mycocin from *Williopsis saturnus* var. *mrakii* inhibits β-1, 3-glucan synthesis (Yamamoto et al. 1986) and the KT28 toxin of *Saccharomyces cerevisiae* inhibits DNA synthesis (Schmitt et al. 1989).

Regardless of the mode of action of mycocins, the first step of cell interaction is binding to the cell wall surface. In contrast to the time-lag process of membrane damage, binding of K1 mycocin to yeast cells is rapid and energy-independent (Al-Aidroos and Bussey 1978, Bussey et al. 1979). Binding is pH-dependent and may be responsible for the pH range of mycocin activity on yeast cells. Binding sites may be particular cell wall receptors, which have other functions, for instance, the uptake of nutrients. These receptors play a role not only in binding but, apparently, in translocation of mycocins through the wall inside the cell where they reach their targets and exert killing action.

Comparison of mycocin killing spectra on whole cells and on spheroplasts showed that the cell wall determines the specificity of mycocin action. *Saccharomyces cerevisiae* toxin K1 can kill cells of the same species and those of *Candida glabrata* (Bussey and Skipper 1976, Young and Yagiu 1978), but it has a wide spheroplast-killing action and can kill the spheroplasts from *Candida albicans, C. utilis, Kluyveromyces lactis* and *Debaryomyces (Schwanniomyces) occidentalis* although the whole cells of these species are insensitive to the toxin (Zhu and Bussey 1989). It has been shown that β-1, 6-glucan is a component of cell-wall receptors for the *S. cerevisiae* mycocin (Hutchins and Bussey 1983) and also for *Hanseniaspora uvarum* mycocin (Radler et al. 1990). Probably, the β-subunit of the K1 mycocin is a lectin-like domain that is essential for recognition and binding (Sturley et al. 1986, Douglas et al. 1988). The mannoprotein of the cell wall binds another mycocin, KT 28, of *S. cerevisiae* (Schmitt and Radler 1987, 1988). Mutations that change the molecular structure of cell wall glucans and mannans alter their capacity to act as receptors, prevent binding of the mycocins and induce resistance to them (Schmitt and Radler 1988, Nakajima et al. 1989). Yeast and hyphal forms of the same organism are different in their sensitivity to mycocins (Golubev and Boekhout 1992) as there are differences in the composition of their walls (Cole and Nozawa 1981).

The early studies of the killer phenomenon were limited to *Saccharomyces cerevisiae* and were devoted to genetic, biochemical and molecular biological aspects, although in the 1970s, mycocinogenic strains were reported in other yeast genera (Philliskirk and Young 1975, Stumm et al. 1977, Kandel and Stern 1979). Until recently there has been little rational basis for selecting the sensitive strains employed and most surveys of the incidence of killer yeasts in culture collections or natural substrates used one or two strains of *S. cerevisiae* and *Candida glabrata.* The frequency of mycocinogenic strains proved low (Middelbeek et al. 1980a, Rosini 1983, Starmer et al. 1987). However, their frequency was higher when screening for killer activity was conducted between strains of the same species, for instance, *S. cerevisiae* (Naumov et al. 1986, Thornton 1986, Heard and Fleet 1987). Similarly, by cross-testing within a species and by using taxonomically related organisms as target cultures, it was found that 98% of 63 strains of *Rhodotorula mucilaginosa* (Golubev and Churkina 1990) and 35% of 62 strains of *Pichia membranifaciens* (Golubev and Blagodatskaya 1993) were mycocinogenic. These data demonstrated the principal trait of mycocins – their specific toxicity. They are active against organisms related to killers, and the choice of sensitive strains for detecting mycocinogenic strains has become a taxonomic problem.

At present, killer activity has been found in about 80 species representing almost 20 genera among both ascomycetous and basidiomycetous yeasts (Wickner 1985, Bruenn 1986, Young 1987) (Table 7). If one takes into account that several types of killers were identified in some species, then to date the number of killer types in yeasts is over 100.

One of the most important conditions for detecting killer activity is the pH of the test medium. Killer activity is expressed under acidic conditions, usually at a pH within the range of 3–6. As a rule, yeast mycocins are most active at pH 4–5 (Woods and Bevan 1968, Young and Yagiu 1978, Middelbeek et al. 1979, Tolstorukov et al. 1989). With some exceptions (Ohta et al. 1984, Vustin et al. 1989), mycocins are rapidly inactivated at increased temperatures, and 15–20°C is the preferred temperature range for incubation during the assay for killer activity. Addition of glycerol (10–15%) to the medium led to broader inhibition zones surrounding *Kluyveromyces* and *Pichia* killers and significantly increased the sensitivity of the bioassay (Lehmann et al. 1987b). Expression of killer activity by halotolerant yeasts is prompted and often enhanced in the presence of increasing (4–12%) NaCl concentrations (Kagiyama et al. 1988, Suzuki et al. 1989). Yeast mycocins are more stable in agar medium than in liquid, and agitation causes their inactivation (Woods and Bevan 1968, Wilson and Whittaker 1989). The concentration of sensitive cells influences the sensitivity of the bioassay; in the case of an abundant lawn of the sensitive strain and a small inoculum of the killer, the inhibition zone can be narrow and rapidly overgrown or not developed at all. In addition, the composition of the medium and buffer solution may contribute to the sensitivity of the assay (Panchal et al. 1985). As a rule, nutritionally rich organic media are more suitable than synthetic media. In most cases, glucose–yeast extract–peptone agar or malt agar with sodium citrate–phosphate

Table 7
Yeast species for which mycocinogenic activity has been reported

Yeast species	Reference	Yeast species	Reference
Candida albicans	Rogers and Bevan (1978)	*P. bimundalis*	Polonelli et al. (1987)
C. dattila	Choi et al. (1990)	*P. cactophila*	Starmer et al. (1987)
C. glabrata	Sriprakash and Batum (1984)	*P. canadensis*	Lehmann et al. (1987a)
C. guilliermondii	Polonelli et al. (1987)	*P. ciferrii*	Nomoto et al. (1984)
C. holmii	Nagornaya et al. (1989)	*P. fabianii*	Polonelli et al. (1987)
C. krusei	Lehmann et al. (1987a)	*P. farinosa*	Suzuki and Nikkuni (1989)
C. maltosa	Polonelli et al. (1987)	*P. guilliermondii*	Zekhnov et al. (1989)
C. neodendra	Suzuki et al. (1989)	*P. holstii*	Polonelli et al. (1987)
C. parapsilosis	Zekhnov et al. (1989)	*P. inositovora*	Hayman and Bolen (1990)
C. pseudotropicalis	Polonelli et al. (1987)	*P. jadinii*	Vaughan-Martini et al. (1988)
C. sonorensis	Starmer et al. (1987)	*P. kluyveri*	Zorg et al. (1988)
C. sp.	Yokomori et al. (1988)	*P. membranifaciens*	Golubev and Blagodatskaya (1993)
C. sphaerica	Vaughan-Martini et al. 1988	*P. mexicana*	Starmer et al. (1987)
C. valida	Young and Yagiu (1978)	*P. minuta* var. *nonfermentans*	Polonelli et al. (1987)
C. versatilis	Vaughan-Martini et al. (1988)	*P. ohmeri*	Zekhnov et al. (1989)
Cryptococcus albidus	Starmer et al. (1987)	*P. opuntiae*	Starmer et al. (1987)
C. laurentii	Golubev and Kuznetsova (1989)	*P. petersonii*	Nomoto et al. (1984)
C. podzolicus	Golubev (1991b)	*P. pini*	Zekhnov et al. (1989)
Cystofilobasidium bisporidii	Golubev (1990b)	*P. quercuum*	Zekhnov et al. (1989)
Debaryomyces carsonii	Polonelli et al. (1987)	*P. spartinae*	Polonelli et al. (1987)
D. hansenii	Suzuki et al. (1989)	*P. subpelliculosa*	Young and Yagiu (1978)
D. polymorphus	Vaughan-Martini et al. (1988)	*P. thermotolerans*	Ganter and Starmer (1992)
D. vanrijiae	Zekhnov et al. (1989)	*Rhodotorula fujisanensis*	Golubev (1992a)
Filobasidium capsuligenum	Golubev and Kuznetsova (1991)	*R. glutinis*	Golubev (1989a)
Hanseniaspora uvarum	Radler et al. (1990)	*R. mucilaginosa*	Golubev and Churkina (1990)
Kloeckera apiculata	Rosini and Cantini (1987)	*R. pallida*	Golubev (1992b)
K. japonica	Starmer et al. (1987)	*Saccharomyces cerevisiae*	Bussey et al. (1990)
Kluyveromyces aestuarii	Vaughan-Martini and Rosini (1989)	*S. paradoxus*	Naumov (1985)
K. dobzhanskii	Vaughan-Martini and Rosini (1989)	*S. unisporus*	Nagornaya et al. (1989)
K. lactis	Stark et al. (1990)	*Sporidiobolus johnsonii*	Golubev and Tsiomenko (1985)
K. lodderae	Vaughan-Martini and Rosini (1989)	*S. pararoseus*	Golubev et al. (1988)
K. marxianus	Lehmann et al. (1987b)	*Trichosporon capitatum*	Morace et al. (1983/84)
K. phaffii	Vaughan-Martini and Rosini (1989)	*Williopsis californica*	Vustin et al. (1988a)
K. wickerhamii	Vaughan-Martini and Rosini (1989)	*W. pratensis*	Vustin et al. (1988b)
K. wikenii	Rosini and Cantini (1987)	*W. saturnus*	Ohta et al. (1984)
Metschnikowia pulcherrima	Vustin et al. (1990)	*W. saturnus (W. beijerinckii)*	Vustin et al. (1988a)
Pichia acaciae	Worsham and Bolen (1990)	*W. saturnus* var. *mrakii*	Yamamoto et al. (1988)
P. amethionina	Starmer et al. (1987)	*W. saturnus* var. *sargentensis*	Lehmann et al. (1987b)
P. anomala	Sawant et al. (1989)	*W. saturnus* var. *subsufficiens*	Vustin et al. (1988a)
P. antillensis	Starmer et al. (1987)		

buffer are used. It is apparent that both the level and expression of mycocinogenic activity depends on a number of variables. In particular, the assay conditions can be crucial for detecting killers with low activity or those organisms that are weakly sensitive.

Yeast mycocins possess only antifungal activity and do not act against bacteria or protozoa. Further, no pharmacological activity was detected in tests with animal organs (Ohta et al. 1984, Pfeiffer et al. 1988). A few reports of the antibiotic action of yeast killers against a wide variety of prokaryotic and eukaryotic organisms resulted from an unwarranted extension of the killer phenomenon to any observed growth inhibition (Polonelli and Morace 1986, Nagornaya et al. 1989). There were

no attempts to characterize the toxic substances cited in these reports and the growth inhibition observed was due to metabolic products other than mycocins. In many cases, the antibacterial activity of yeasts is caused by pH changes in the medium as a result of organic acid production or the selective uptake and exchange of ions. In respect to antifungal activity, it is necessary to distinguish the killer phenomenon from other inhibition effects, for example, arresting of growth from mating pheromones. The broad anti-yeast activity of fresh *Metschnikowia pulcherrima* isolates (Vustin et al. 1990) is probably associated with excretion of the iron-binding agent pulcherriminic acid.

3. Taxonomic implications of sensitivity to mycocins

It is well known that fungal cell walls are diverse in architecture and chemical composition, and some of these differences are of taxonomic importance (Bartnicki-Garcia 1968, Kreger-van Rij and Veenhuis 1971a, Fleet 1985, Weijman and Golubev 1987). Taking into account the proteinaceous nature of mycocins and the involvement of different cell wall components in their binding, it is reasonable to deduce that mycocin activity against whole cells may be restricted to taxonomically related organisms and that killer-sensitive interactions reflect taxonomic affiliation. Present evidence supports such a view although much experimental data need to be thoroughly examined as most workers engaged in research on the killer phenomenon do not consider taxonomic problems.

The main obstacle that delayed recognition of taxonomic specificity of mycocin action is the varying differences in host ranges. Although all mycocins are active against organisms taxonomically related to killers, the degree of relatedness may vary from strains of the same species to species of closely related genera or even higher taxa. Apparently, the diversity of cell-wall receptors involved in binding of mycocins can be both unique and common for some taxa and may be the basis of differences in mycocin action ranges. Until now, the primary functions of these receptors was not established in yeasts.

4. Taxonomic implications among the ascomycetous yeasts

Presently available information on killing patterns of mycocins produced by ascomycetous yeasts is extremely fragmentary and none of the screenings are wholly comprehensive. The sets of organisms used as target cultures are usually very limited and were not selected with a taxonomic perspective. As mentioned above, the K1 mycocin of *Saccharomyces cerevisiae* can kill the cells of the same species and *Candida glabrata*. Tolstorukov et al. (1989) reported that K1 mycocin is active against *Candida fluviatilis, C. freyschussii* and *C. rugosa*, and the K2 mycocin of *S. cerevisiae* has a broad killing spectrum which additionally includes *Kluyveromyces marxianus, Pichia canadensis, Candida maltosa* and *C. tropicalis*. The mycocins produced by *Kluyveromyces* spp. are active not only against species of this genus and their anamorphs (*Candida kefyr, C. pseudotropicalis, C. sphaerica*), but also inhibit the growth of a rather diverse group of yeasts (*Pichia anomala, S. cerevisae, Zygosaccharomyces rouxii, Candida albicans, C. glabrata, C. intermedia, C. utilis* and *Kloeckera apiculata*) (Panchal et al. 1985, Lehmann et al. 1987a, Rosini and Cantini 1987). Vaughan-Martini and Rosini (1989) studied intrageneric killer-sensitive interactions in *Kluyveromyces*, and concluded that sensitivity is not a species-specific property, but is strain-related. However, such an interpretation did not consider a serious limitation of mycocin-sensitivity testing by simple plate assay, namely, it does not allow one to distinguish between immunity and resistance to mycocins. These two types of insensitivity differ fundamentally, both in their mechanisms and taxonomic significance. In *S. cerevisiae* the mycocins, or their precursors, contain a component which gives immunity (specific for only one mycocin type) to killer strains. Killers, when cured of the extrachromosomal genome which produces the mycocin, became sensitive to its action. Immunity appears to be conferred at the cytoplasmic membrane level and this component may act as a competitive inhibitor of mycocin by saturating membrane receptors (Boone et al. 1986, Hanes et al. 1986, Douglas et al. 1988). The so-called neutral strains contain the genetic determinants for mycocin synthesis, either the mycocin is produced in an inactive form or it is not secreted. Consequently, such strains retain immunity (Bussey et al. 1982, Wingfield et al. 1990). Thus, the insensitivity caused by immunity is a clone-related property, which, when coupled with possible cross-immunity between the killers and neutral strains-producing immunologically similar mycocins, may interfere with resistance shown at the cell-wall level and conferred by the nuclear genotype. All these reasons taken together can give a complicated picture of killer-sensitive relationships. For example, the study of the genus *Kluyveromyces* demonstrated that more than half of the strains examined were of killer and neutral phenotypes (Vaughan-Martini and Rosini 1989). The preceding observations are the major reasons why many authors consider the sensitivity patterns to be a strain-specific property and propose to use them as a fingerprinting tool to biotype strains of a species. In respect to closely related organisms, the responses to mycocins that are free from the effects of immunity are of taxonomic interest. In such instances, it was found that all *Kluyveromyces yarrowii* strains (which are a sensitive phenotype) have identical responses to *Kluyveromyces* mycocins. Interestingly, the strains of *K. marxianus (K. fragilis)* and *Candida pseudotropicalis* have the same sensitivity patterns but differ from *C. kefyr*, a synonym. These data are consistent with electrophoretic karyotypes, antigenic reactions, and long-chain fatty acid profiles (Caretta et al. 1980, Viljoen and Kock 1989a).

Many species in the genera *Pichia* and *Williopsis* produce mycocins (Table 7). No representatives of

Table 8
Species susceptible to mycocins produced by sporidiobolaceous yeasts

Species	Susceptibility[a]			Species	Susceptibility[a]		
	S.john	S.para	R.glut		S.john	S.para	R.glut
Bensingtonia ingoldii			+	*R. fujisanensis*		+	+
B. intermedia	+		+	*R. glutinis*		+	+
B. miscanthi			+	*R. graminis*			+
B. yuccicola			+	*R. hordea*	+		+
Leucosporidium antarcticum	+		+	*R. hylophila*			+
L. fellii	+			*R. ingeniosa*	+	+	+
L. scottii	+		+	*R. javanica*	+		+
Rhodosporidium babjevae			+	*R. lignophila*			+
R. diobovatum		+	+	*R. mucilaginosa*		+	+
R. fluviale		+	+	*R. muscorum*			+
R. kratochvilovae		+	+	*Sporidiobolus johnsonii*	+	+	+
R. lusitaniae			+	*S. microsporus*	+		+
R. malvinellum			+	*S. pararoseus*		+	+
R. paludigenum	+	+	+	*S. ruineniae*			+
R. toruloides		+	+	*Sporobolomyces alborubescens*			+
Rhodotorula araucariae	+	+	+	*S. falcatus*	+		
R. bacarum			+	*S. roseus*		+	+
R. fragaria		+	+	*S. singularis*			+

[a] Abbreviations: S.john, *Sporidiobolus johnsonii*; S.para, *Sporidiobolus pararoseus*; R.glut, *Rhodotorula glutinis*

these genera, except for *W. (Zygowilliopsis) californica* (Nomoto et al. 1984), could be cured of their killer activity and none of the species had detectable dsRNA or DNA plasmids. The observations suggest that mycocin production in these species is determined by nuclear genes. Most mycocins produced by *Pichia* and *Williopsis* are heat stable but are protease-sensitive and function over a wide pH range (Ashida et al. 1983, Ohta et al. 1984, Vustin et al. 1989). They have broad activity and kill species in the ascosporogenous genera *Saccharomycopsis (Arthroascus), Debaryomyces (Schwanniomyces), Kluyveromyces, Saccharomyces, Zygosaccharomyces, Pichia, Williopsis (Zygowilliopsis)* and *Saccharomycodes.* By contrast, members of the genera *Clavispora, Hanseniaspora, Metschnikowia, Torulaspora, Williopsis* and *Schizosaccharomyces* were resistant to these mycocins (Kagiyama et al. 1988, Sawant et al. 1988, Vustin et al. 1988a, Suzuki et al. 1989). It should be noted that representatives of the genus *Candida* are heterogeneous with respect to their sensitivity patterns (Yamamoto et al. 1988, Vustin et al. 1988b, Tolstorukov et al. 1989) and species of basidiomycetous affinity are insensitive to the mycocins produced by ascomycetous yeasts. *Williopsis pratensis* is an exception. Its mycocin is active not only against *Debaryomyces hansenii, D. (Schwanniomyces) occidentialis, W. (Zygowilliopsis) californica* but also against some basidiomycetous yeasts, namely, sporidiobolaceous species (Vustin et al. 1991). In the genus *Pichia, P. acaciae* and *P. inositovora* differ both in genetic determination of killer phenotype (see above) and apparently in the limited ranges of organisms susceptible to their mycocins (Worsham and Bolen 1990, Hayman and Bolen 1990).

5. Taxonomic implications among the basidiomycetous yeasts

With the exception of *Cryptococcus humicola* (Golubev and Shabalin 1994), none of the mycocins produced by basidiomycetous yeasts are active against ascosporogenous yeasts. Moreover, the host ranges of *Rhodotorula* and *Sporidiobolus* mycocins include only organisms classified in the family Sporidiobolaceae (Table 8) whereas mycocins of *Cryptococcus, Cystofilobasidium* and *Filobasidium* kill members of the order Tremellales including the Filobasidiaceae (Table 9). As a rule, sporidiobolaceous yeasts are insensitive to mycocins produced by tremellaceous species as well as the reverse. Recently, it was found that although *Rhodotorula fujisanensis* mycocin mainly acts against sporidiobolaceous yeasts, it also shows a weak activity against some strains of tremellaceous yeasts (Golubev 1992a). These differences between sporidiobolaceous and tremellaceous yeasts in sensitivity and killing patterns of their mycocins correlates with the whole range of important taxonomic and phylogenetic markers, for example septal pore ultrastructure (Moore 1987b), monosaccharide composition of capsular polysaccharides (Weijman and Golubev 1987), and sequence similarity of small (Gottschalk and Blanz 1985) and large subunit

Table 9
Host ranges of mycocins produced by tremellaceous yeasts

Species	Susceptibility[a]				Species	Susceptibility[a]			
	C.pod	F.cap	C.bis	C.lau		C.pod	F.cap	C.bis	C.lau
Bullera armeniaca	+		+	+	*C. skinneri*	+	+	+	+
B. crocea	+		+	+	*C. terreus*	+	+	+	+
B. dendrophila	+			+	*C. tyrolensis*		+	+	+
B. globispora			+	+	*C. vishniacii*		+	+	+
'*B. grandispora*'			+		*Cystofilobasidium bisporidii*			+	
B. miyagiana	+	+		+	*C. capitatum*			+	+
B. oryzae		+		+	*C. infirmominiatum*			+	+
B. pseudoalba	+	+		+	*Fellomyces fuzhouensis*		+	+	+
B. sinensis				+	*F. horovitziae*		+		+
B. variabilis	+	+	+	+	*F. penicillatus*		+	+	
Bulleromyces albus	+		+	+	*F. polyborus*		+	+	
Cryptococcus aerius	+	+	+	+	*Fibulobasidium inconspicuum*	+			
C. albidosimilis			+	+	*Filobasidiella neoformans*	+	+	+	+
C. albidus		+	+	+	*Filobasidium capsuligenum*	+	+	+	+
C. amylolentus		+	+	+	*F. floriforme*			+	+
C. antarcticus			+	+	*F. uniguttulatum*			+	+
C. baldrensis			+	+	*Kockovaella thailandica*		+	+	
C. consortionis		+	+	+	*Mrakia frigida*				+
C. curiosus				+	*M. nivalis*				+
C. dimennae			+	+	*M. stokesii*				+
C. flavus				+	*Sebacina penetrans*	+	+		+
C. friedmannii			+	+	*Sirobasidium magnum*	+		+	+
C. fuscescens	+	+	+	+	*Sterigmatosporidium polymorphum*	+			+
C. gastricus	+	+	+	+	*Tremella aurantia*	+	+	+	+
C. gilvescens			+	+	*T. encephala*		+	+	
C. hempflingii		+	+	+	*T. foliacea*	+	+	+	+
C. huempii			+	+	*T. mesenterica*		+		
C. heveanensis	+		+	+	*T. mycophaga*	+	+	+	+
C. humicolus			+		*T. simplex*	+	+	+	+
C. hungaricus	+		+	+	*Trichosporon pullulans*				+
C. kuetzingii			+	+	*Tsuchiyaea wingfieldii*		+	+	
C. laurentii	+	+	+	+	*Udeniomyces megalosporus*			+	+
C. lupi			+	+	*U. pyricola*			+	+
C. luteolus				+	*U. puniceus*			+	+
C. macerans			+	+	*Xanthophyllomyces dendrorhous*			+	+
C. magnus			+	+					
C. marinus		+	+						
C. podzolicus	+	+	+	+					

[a] Abbreviations: C.pod, *Cryptococcus podzolicus*; C.bis, *Cystofilobasidium bisporidii*; C.lau, *Cryptococcus laurentii*.; F.cap, *Filobasidium capsuligenum*.

rRNAs (Fig. 16). Similar to the activity found for ascomycetous mycocins, basidiomycetous mycocins generally have no fixed taxonomic level for their action. In practice, this feature dictates that the use of each mycocin as a taxonomic tool must be preceded by a careful study of the killing pattern. Owing to different host ranges, mycocins may serve to resolve different levels of taxonomic organization. Broad-spectrum mycocins are apparently of more interest for overall phylogenetic evaluations, whereas narrow-spectrum mycocins may be used for clarification of the taxonomy of some closely related organisms.

The mycocin produced by *Rhodotorula pallida* has the narrowest known range of action; only strains of this species and *R. minuta* are affected by its action (Golubev

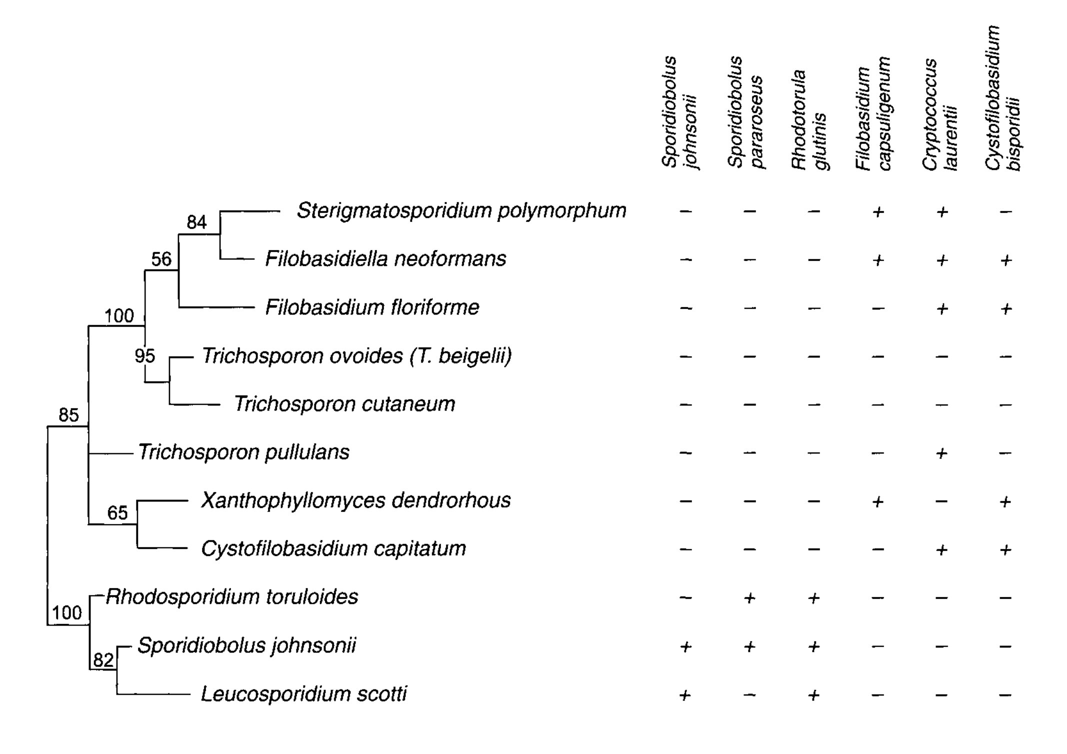


Fig. 16. Similarity of basidiomycetous taxa from partial 18S and 26S rRNA sequences (phylogenetic tree with bootstrap values recalculated by C.P. Kurtzman from the data of Guého et al. 1989), and the sensitivity (+) or insensitivity (–) of the species to mycocins produced by *Sporidiobolus johnsonii* (Golubev and Tsiomenko 1985), *S. pararoseus* (Golubev et al. 1988), *Rhodotorula glutinis* (Golubev 1989a), *Filobasidium capsuligenum* (Golubev and Kuznetsova 1989), and *Cystofilobasidium bisporidii* (Golubev 1990b).

1992b). Sensitive cultures show decreased budding, but cell viability is not affected. The mycocins secreted by *Sporidiobolus johnsonii (Sporobolomyces salmonicolor)* act mainly against species of *Sporidiobolus* and its anamorphs (Golubev and Tsiomenko 1985). The killing patterns from *Sporidiobolus pararoseus* (Golubev et al. 1988) and *Rhodotorula glutinis* are wider (Table 8). Yeast phases of smut fungi of the genera *Microbotryum, Sphacelotheca* and *Ustilago* are sensitive to *R. glutinis* mycocin (Golubev 1989a). The mycocin produced by the type strain of *Cryptococcus laurentii* has an inhibitory effect on yeast phases of the genera *Atractogloea, Itersonilia, Microstroma* and *Trimorphomyces* (Golubev and Kuznetsova 1989). Except for *Trimorphomyces syzygius,* these taxa are sensitive to *Cystofilobasidium bisporidii* mycocin. In addition, the latter acts against species in *Calocera, Guepiniopsis* and rust fungi of the genera *Puccinia* and *Gymnosporangium* (Golubev 1990b). According to our data, these rust fungi assimilate D-glucuronate, produce xylose-containing exopolysaccharides, have ubiquinones Q-8, 9, 10 (Sugiyama et al. 1988) and show high sequence homology in their 5S rRNA when compared with tremelloid yeasts (Gottschalk and Blanz 1985). Although this evidence supports the proposal that the Uredinales are the ancestral group which gave rise to the auricularioid and tremelloid fungi (Linder 1940), it is now believed that the yeast stages of the rust fungi that were studied represent contaminating tremelloid yeasts (P.A. Blanz, unpublished data, chapter 10).

In many cases, all strains of the same species and closely related species of the same genus have identical responses to specific mycocins. However, some taxa are heterogeneous not only within a genus but also within a species. Several reasons for such heterogeneity were mentioned above (immunity of killer and neutral strains, mutants with modified cell-wall components), but there is one more important reason – the taxonomic heterogeneity of many yeast taxa. As a rule, the teleomorphic taxa (taking into account immunity) are homogeneous in this respect in contrast to the anamorphic species in which the heterogeneity in sensitivity patterns occurs much more frequently. For example, almost all strains of *Sporidiobolus* spp. are sensitive to *Rhodotorula glutinis* mycocin while the ballistosporogenous genera *Sporobolomyces* and *Bensingtonia* include both sensitive (Table 8) and resistant species *(S. gracilis, S. lactophilus, S. oryzicola, S. sasicola, S. subbrunneus, B. naganoensis* and *B. subrosea).* The reassignment of basidiomycetous *Candida* spp. to *Cryptococcus* and *Rhodotorula* (Weijman et al. 1988) resulted in higher heterogeneity of these anamorphic genera. The sensitivity to *Rhodotorula glutinis* and/or *R. mucilaginosa* mycocins supports the transfer of the species *Candida fragariorum, C. graminis, C. hylophila, C. ingeniosa, C. javanica, C. lignophila, C. muscorum, C. sonckii* and *Cryptococcus*

phylloplanus to the genus *Rhodotorula*. However, the species *Candida acuta, C. auricularia, C. bogoriensis, C. buffonii, C. diffluens, C. foliorum, C. philyla* and *C. pustula* are insensitive to the *Rhodotorula* mycocins. Among recently proposed combinations, *Cryptococcus amylolentus, C. hungaricus, C. marinus* and *C. podzolicus* are sensitive to *Filobasidium capsuligenum* and/or *C. podzolicus* mycocins (Table 9) but *Candida aquatica, C. curiosa, C. huempii, C. tsukubaensis* and *Cryptococcus yarrowii* are not. In contrast to typical cryptococci, the latter, besides containing xylose, also have fucose and rhamnose in their exocellular polysaccharides (Golubev and Blagodatskaya 1978, Roeijmans et al. 1989). Unlike *Sterigmatomyces* and *Kurtzmanomyces*, species of *Fellomyces* and *Sterigmatosporidium* are sensitive to mycocins produced by tremellaceous yeasts and this observation is in keeping with relationships determined from rRNA sequence comparisons (Guého et al. 1990).

Much of the preceding data suggest that killer-sensitive relationships reflect phylogenetic relatedness between yeasts. The presence of immunity to killer toxins and possible occurrence of resistant mutants limits the value of mycocin sensitivity for identification. However, the killer phenomenon may be an additional taxonomic tool with which to examine yeast classification.

Chapter 9

Nuclear DNA hybridization: Quantitation of close genetic relationships

C.P. Kurtzman

Contents

1. Introduction

The definition of a species, whether of higher plants, animals, or microorganisms, has as its basis the principle of genetic isolation. Simply put, members of a species are considered interfertile, whereas genetically separate species are not (Dobzhansky 1976). However, until the late 1960s, the primary means for defining species of yeasts lay with the subjective appraisal and weighting of phenotypic properties such as cellular morphology and growth responses on various sugars and other compounds. Although it was known that the assimilation and fermentation of many compounds were controlled by one or only a few genes (Lindegren and Lindegren 1949, Winge and Roberts 1949) and that using growth reactions could result in an erroneous definition of species, practical genetic means for defining species often did not exist. With the development of methods for measuring DNA duplex formation came the opportunity to define species on the basis of overall genetic similarity. When measurements of DNA reassociation are used to define species, the extent of DNA relatedness expected between members of a biological species must first be determined. In the discussion that follows, various aspects of this issue will be examined.

DNA relatedness studies have had a major impact on yeast systematics, but in this era of increasingly sophisticated molecular techniques that often involve gene sequencing, it is sometimes forgotten that DNA hybridization has provided the framework upon which many other types of molecular genetic comparisons have evolved. For this reason, results from DNA reassociation are considered the "gold standard" by many because they provide the first approximation of whether or not strains are members of the same biological species.

2. Cellular DNAs

Several classes of DNA occur in eukaryotes, and their presence affects estimates of relatedness between taxa since it is the intent of most studies to measure genomic similarity from complementarity of single-copy nuclear DNA (nDNA) sequences. Christiansen et al. (1971) reported up to 15% rapidly renaturing sequences in the nDNA of yeasts, which may be interpreted as analogous to the repeated sequences found in higher eukaryotes (Britten and Kohne 1968). However, the presence of rapidly renaturing, plasmid-like 2 µm circular DNA in *Saccharomyces cerevisiae* (Bak et al. 1972) with a buoyant density similar to nDNA suggests the proportion of repeated sequences in nDNA to be smaller than first thought. Similar circular DNA species have been reported from *Pichia canadensis (Hansenula wingei)* and *Candida glabrata* (O'Connor et al. 1975, 1976). Linear DNA plasmids have been discovered in *Kluyveromyces lactis* and many other species (Gunge et al. 1981, Fukuhara 1995). These plasmids, which are lighter than the densities of both nuclear and mitochondrial DNA, appear to be associated with killer characters of yeasts. The work of Timberlake (1978) indicates the genome of the fungus *Aspergillus nidulans* to consist of approximately 97–98% unique sequences and 2–3% repeated sequences. The repeated sequences appear to code for ribosomal RNA.

Nuclear DNA represents the preponderance of cellular DNA in yeasts, and it has a kinetic complexity of 6.5–14×10^9 daltons or about two to five times that found in *Escherichia coli* (Christiansen et al. 1971, Petes et al. 1973). Between 5 and 20% of the total DNA in *S. cerevisiae* is mitochondrial in origin. It is present in a variable number of copies and is arranged in closed circles with a G+C content noticeably lower than the nDNA (Hartwell 1974). Sedimentation studies (Blamire et al. 1972) and measurements of contour length (Hollenberg et al. 1970, Petes et al. 1973) suggest a molecular mass of 46×10^6 daltons. The mitochondrial DNA (mtDNA) contour length for several other yeasts has been reported to be 25–50% of that of *S. cerevisiae* (O'Connor et al. 1975, 1976, McArthur and Clark-Walker 1983).

3. DNA isolation and purification

Methods for isolation and purification of yeast DNA were previously described in detail and the following account will serve only as a brief review to direct attention to pertinent references. Most methods for DNA reassociation

require substantial amounts of DNA, and it is not uncommon to process 5–50 g (wet weight) of cells.

For most yeast strains, DNA isolation is relatively straightforward. Cell wall breakage may be effected by mechanical or lytic means. Mechanical methods have the advantage of being relatively rapid but, unless cell breakage is carefully monitored, considerable shearing of the DNA may result. The Braun cell homogenizer is one of the more frequently used mechanical devices, and causes breakage by shaking of buffered cell suspensions in the presence of 0.45 mm glass beads (Bak and Stenderup 1969, Price et al. 1978, Kurtzman et al. 1980a). An inexpensive variation of this technique involves placing the buffered cell and bead mixture into a test tube and "vortexing" the mixture (Lipinski et al. 1976, Van Etten and Freer 1978). Less frequently reported methods include grinding acetone-dried cells in a ball mill (Martini and Phaff 1973) and grinding cells in liquid nitrogen with a mortar and pestle (Storck and Alexopoulos 1970, Mendonça-Hagler et al. 1974). Sonication is seldom used for cell breakage because it is inefficient for yeasts and it causes extreme and uncontrolled DNA shearing.

Lytic methods offer the possibility of isolating DNA with a higher molecular weight than might be obtained by mechanical cell disruption. Meyer and Phaff (1969) and Bicknell and Douglas (1970) used a modification of the Smith and Halvorson (1967) technique in which cells undergo autolysis in the presence of saline containing EDTA (ethylenediamine tetraacetate), 2-mercaptoethanol and sodium dodecylsulfate. The commercial enzyme preparations Glusulase and Zymolyase 100T may be used to prepare osmotically fragile sphaeroplasts by cell wall digestion. Following this step, high molecular-weight DNA is isolated from the lysed sphaeroplasts. Cryer et al. (1975) have given details of these procedures. Zymolyase 100T generally promotes more rapid cell-wall dissolution than Glusulase, but neither enzyme preparation is particularly effective with basidiomycetes. Rhodes and Kwon-Chung (1982), however, reported dissolution of *Cryptococcus neoformans* cell walls with the multienzyme preparation Mutanase.

Following rupture or lysis of the cells, DNA isolation and purification may be carried out by the methods of Marmur (1961) and Bernardi et al. (1970) as detailed by Price et al. (1978). Basically, this consists of making the buffered cell suspension 1 M in sodium perchlorate and 1% in sarcosate and emulsifying with an equal volume of chloroform/iso-amyl alcohol (3-methylbutan-1-ol) (24:1, v/v). After incubation and phase separation by centrifugation, DNA is precipitated from the aqueous phase with cold ethanol. The DNA is treated with a combination of α-amylase and pancreatic ribonuclease (RNase), and finally with Pronase. Following enzyme removal, the DNA is again precipitated with ethanol, spooled, redissolved and further treated with α-amylase, pancreatic RNase and T_1 RNase. Additional purification can include hydroxyapatite fractionation and/or banding in a cesium chloride gradient.

Some yeasts have active deoxyribonucleases (DNases) that escape denaturation during the usual extraction procedures and cause sufficient degradation of the DNA to prevent spooling. Several methods have produced various degrees of success for deactivating nucleases and merit testing if problems are encountered. Diethyl pyrocarbonate, used at 0.5–1.0% at the time of cell breakage, gave encouraging results in studies of *Lipomyces* spp. (D.L. Holzschu and H.J. Phaff, personal communication) and *Sporidiobolus* spp. (Holzschu et al. 1981). Ehrenberg et al. (1976) discussed the interaction of diethyl pyrocarbonate with nucleic acids and nucleases and emphasized some of the precautions to be taken. Allergy to the compound may be a problem for some individuals. Kurtzman et al. (1986) found that the spermine/spermidine/sucrose buffer of Morris, as cited by Timberlake (1978), was much more effective at inactivating nucleases in *Aspergillus* spp. and *Neurospora* spp. than the sucrose buffer frequently used in yeast studies (Price et al. 1978). This was confirmed by Guého et al. (1984, 1985) for species of *Geotrichum, Dipodascus* and *Trichosporon.* Methods for assessing DNA purity include chemical analyses as well as spectrophotometric measurements and have been summarized by Price et al. (1978) and Johnson (1981).

4. DNA base composition

4.1. Methodology

Base composition of nuclear and mitochondrial DNA, expressed as molar percentages of guanine-plus-cytosine (G+C), can be determined from thermal denaturation profiles (melts), buoyant density in cesium salt gradients generated by ultracentrifugation, chemical analysis, absorbance ratios, or high-performance liquid chromatography (HPRL) of nucleotides or free bases (Johnson 1981, Hamamoto et al. 1986b). Many investigators use thermal denaturation (Marmur and Doty 1962), but Bak (1973) showed that results obtained by this method can be greatly affected by sample impurities and/or minor DNA species and must therefore be interpreted with caution. Cesium chloride buoyant density determinations (Schildkraut et al. 1962) generally show the greatest accuracy since they are unbiased by the presence of contaminating RNA, mtDNA and other impurities such as carbohydrates and proteins.

4.2. Taxonomic uses of guanine-plus-cytosine (G+C) values

The taxonomic uses of G+C values are mainly exclusionary, because the 800 or so yeast species range in nuclear G+C contents from approximately 27–70 mol%, and overlap between unrelated species is inevitable. For example, *Zygosaccharomyces mrakii* and *Z. florentinus* have nuclear G+C contents of 42.2 and 42.4%, respectively, yet they show only 6.0% base sequence complementarity (Price et al. 1978). Since similar G+C contents do not

necessarily mean similar base sequences in the DNA, it must be determined how dissimilar G + C values need to be to state that two strains represent different species. This depends to some extent upon the method used for determination of base composition. When buoyant density is used, the span of G + C values among strains of a species is usually less than 1% (Price et al. 1978, Kurtzman et al. 1980a,b, Phaff et al. 1985), which is approximately coincident with the accuracy of the method, i.e., a standard deviation of 0.00–0.50 mol%. Thermal denaturation shows greater variation, and strains of a species may exhibit a G + C range of as much as 2% (Meyer et al. 1978). Thus, strains showing a difference in G + C contents in excess of 1.0–1.5% by buoyant density or 2.0–2.5% by thermal denaturation may be expected to represent different species.

The nuclear G + C content of ascomycetous yeasts is about 27–50%, whereas that of basidiomycetous yeasts is approximately 50–70%. Except for the narrow range of 48–52%, where some overlap occurs, the taxonomic affinity of anamorphs can be reliably determined from their base composition. For species in the doubtful range, placement can be made by examining cell wall structure under the transmission electron microscope (Kreger-van Rij and Veenhuis 1971a) or through use of the diazonium blue B test (van der Walt and Hopsu-Havu 1976, Hagler and Ahearn 1981). The range of G + C contents among species within a genus is quite often 10% or less as found in *Debaryomyces, Hanseniaspora, Issatchenkia, Kluyveromyces, Metschnikowia* and several other genera. Genera showing a range among species of greater than 10% may be polyphyletic, but a narrower range does not ensure monophyly.

5. DNA relatedness

5.1. Methodology

The methods for assessing DNA relatedness vary, but short of sequencing, they rely on measuring the extent and stability of renatured DNA strands from test pairs, i.e., the fidelity of complementary base pairing. The theoretical and operational aspects of DNA hybridization were elegantly presented by Britten and Kohne (1968), and their paper has served as a primer for those now using DNA reassociation techniques. Depending upon the method, the DNA may or may not need to be labelled with radioisotopes. An interesting variation of the DNA–DNA hybridization technique was employed by Bak and Stenderup (1969). Radioactive complementary RNA was synthesized *in vitro* with each test DNA serving as a template, and the relatedness between taxa was then assessed from hybridization of single-stranded DNA with the synthesized RNA.

In vivo labeling of DNA is frequently done using ^{14}C, ^{3}H or ^{32}P. Each isotope has its particular advantages and disadvantages. The DNA may also be labelled *in vitro* with ^{125}I (Commerford 1971, Mendonça-Hagler and Phaff 1975, Holzschu et al. 1979) or by nick translation (Kelly et al. 1970, Maniatis et al. 1975). DNA labelled with ^{125}I *in vitro* must be highly purified to completely remove RNAs, proteins and carbohydrates. Banding the DNA in cesium chloride in a preparative ultracentrifuge is usually adequate. Following labeling, DNA reassociation reactions may be carried out using the membrane method which involves immobilizing single strands of one of the DNA species onto nitrocellulose filters and allowing sheared single strands of the second DNA to react with the immobilized DNA (Denhardt 1966). Alternatively, both DNA species may be allowed to react in free-solution, and the extent of reassociation assessed by the extent of binding of the resulting duplexes to hydroxyapatite (Brenner 1973) or from their resistance to hydrolysis by S_1 nuclease (Crosa et al. 1973). Price et al. (1978), Johnson (1981), Jahnke (1987) and Kurtzman (1993b) provided references and detailed procedures for these methods.

Besides following DNA reassociation with radioactive preparations, the reactions may also be monitored spectrophotometrically by measuring the kinetics of duplex formation. This technique has been refined to the point where results are comparable to data obtained using isotopes (Subirana and Doty 1966, De Ley et al. 1970, Seidler and Mandel 1971, Kurtzman et al. 1980a).

Another non-isotopic method that shows promise is based on chemiluminescence (Adnan et al. 1993). Single-stranded target DNA is covalently linked in the wells of special microtitre plates and hybridized with photobiotin-labelled probe DNA. Following incubation, extent of reassociation is determined with a chemiluminescent reagent. Sugita et al. (1994) reported good correlation with the spectrophotometric method. Because spectrophotometers with programmable thermal control are not presently being manufactured, chemiluminescence may be a reliable non-isotopic alternative.

A number of factors affect DNA renaturation reactions and so may have a marked effect on the results obtained. Highly purified DNA preparations are essential. Contaminating protein or RNA can result in falsely high or low relatedness values depending on whether the contaminants act as single-stranded or double-stranded DNA in the assay system (Brenner 1973).

If comparisons of taxa are to be made from single-copy nDNA, it is necessary to remove mtDNA, plasmid-like circular DNA and repeat sequence nDNA. Circular plasmid-like DNA as well as circular mtDNA may be relatively easily separated from nDNA by centrifugation in cesium chloride in the presence of ethidium bromide. Williamson and Fennell (1975) emphasized that resolution is poor should mtDNA break into linear fragments during isolation, a not uncommon event. However, they point out that linear mtDNA may be effectively resolved in cesium chloride gradients when treated with 4′,6-diamidino-2-phenylindole (DAPI), a fluorescent dye that preferentially binds with AT-rich DNA. Repeated sequences may be

removed from nDNA by partial reassociation of sheared DNA fragments to a C_0t value of 0.1–0.2 (C_0t is the initial nucleotide concentration in moles per liter multiplied by the time in seconds; Britten and Kohne 1968) followed by fractionation with hydroxyapatite (Price et al. 1978). Because the rate of reassociation is affected by concentration, multiple copies duplex most rapidly and can be removed because of their ability to bind to hydroxyapatite (Britten and Kohne 1968). Price et al. (1978) and Fuson et al. (1979) used this method to remove repeated sequences from yeast DNA for taxonomic studies. The fractionation undoubtedly also removes mtDNA and plasmid-like DNA because both occur as multiple copies and their small molecular weight would allow rapid reassociation when in significant concentration. The same effect may be obtained in spectrophotometric determinations of relatedness if the time between melting and cooling to the reassociation temperature ($T_m - 25$) is not too rapid. Under the conditions used by Kurtzman et al. (1980a), a cooling period of 12 min allowed duplexing of most repetitive nDNA sequences as well as those of mtDNA. Relatedness values of strains common to the work of Price et al. (1978) and of Kurtzman et al. (1980a) were essentially identical, showing that similar results can be obtained with rather different methodologies.

For most studies, DNA fragment size and the ionic strength of the incubation medium may be maintained at any constant value within a fairly wide range without significantly affecting the results (Brenner 1973). However, increasing ionic strength increases the reaction rate. Seidler et al. (1975) and Kurtzman et al. (1980a) used relatively high concentrations of standard saline citrate (SSC, 150 mM sodium chloride/15 mM sodium citrate, pH 7.0) when assessing DNA relatedness spectrophotometrically in order to complete the determination within a few hours. Thus, when C_0t values are used as a measure of the extent of reassociation, the ionic strength of the reaction mixture must be known if values obtained in different studies are to be compared. For example, the incubation mixture used by Kurtzman et al. (1980a) allowed DNA reassociation to proceed nearly five times faster than that which occurred in the buffer system employed by Price et al. (1978). As noted in the definition of C_0t values, initial nucleotide concentration also affects the reaction rate; consequently, a solution containing 100 μg of DNA per ml reassociates twice as fast as one containing 50 μg.

Optimal DNA reassociation occurs at 25° to 30°C below the midpoint of the melting curve (T_m), and this is dependent on base composition since GC base pairs with their triple hydrogen bonds have greater thermal stability than do AT pairs with their double hydrogen bonds (Marmur and Doty 1962, Marmur et al. 1963). Assuming that two yeast DNAs have melting temperatures of 85°C and thus the optimum reassociation temperature ($T_m - 25$) is 60°C, pairs that are highly related at 60°C also show high relatedness at more stringent temperatures such as 65°C or at 75°C. Pairs showing only moderate relatedness at 60°C show much less binding at higher temperatures than closely related pairs, reflecting the greater mismatch of base pairs present. These studies point out the need for proper reannealing temperatures, and also show that higher incubation temperatures allow an estimate of stability that indicates the extent of base pair mismatch. Temperatures below $T_m - 25$ would show even greater reassociation for sparingly related pairs. Relatedness may also be estimated from the thermal stability of the renatured heterologous DNA as compared with renatured homologous DNA. This may be done spectrophotometrically by monitoring thermal melts or with labelled DNA by thermal elution from hydroxyapatite columns (Ullman and McCarthy 1973, Lachance 1980), or by the batch method of Brenner et al. (1969) as modified by Price et al. (1978). Brenner et al. (1972) equated one degree of difference in thermal stability with 1% mismatch in base pairs. Lower thermal stability (ΔT_m) usually correlates well with lower DNA complementarity.

6. Interpretation of DNA relatedness

6.1. Quantitation of relatedness

Measurements of DNA complementarity are commonly expressed as percent relatedness. This usage can be misleading because DNA strands must show at least 75–80% base sequence similarity before duplexing can occur and a reading is registered on the scale of percent relatedness (Bonner et al. 1973, Britten et al. 1974). As seen from the preceding discussion, experimental conditions greatly influence extent of duplex formation. Although replicate determinations often vary by 5%, different methods of measuring DNA relatedness do give essentially the same result. Consequently, when measured under optimum conditions, percent DNA relatedness provides an approximation of overall genome similarity between two organisms. However, the technique does not detect single gene differences or exact multiples of ploidy, but aneuploidy can often be detected (Vaughan-Martini and Kurtzman 1985).

6.2. Correlation with biological relatedness

On the basis of extensive comparisons between species of enteric bacteria, Brenner (1973) proposed that strains showing 70% or greater DNA relatedness should be considered members of the same species. Johnson (1973) compared numerous anaerobic bacteria, and arrived at essentially the same guidelines. Price et al. (1978) examined yeast species in four genera and proposed that strains having 80% or greater DNA relatedness were conspecific, an opinion expressed earlier by Martini and Phaff (1973) from their studies of *Kluyveromyces* spp. From studies of divergence among biotypes of the *Cryptococcus vishniacii* complex, Baharaeen et al. (1982) suggested that strains showing 60% or greater DNA relatedness were conspecific. The preceding guidelines concerning relatedness were based on the observation that strains of

Table 10
Correlation of mating reaction and nuclear DNA complementarity among closely related heterothallic ascomycetous and basidiomycetous yeasts

Species[a]	Mating reaction	DNA rel. (%)	Reference
Filobasidiella neoformans × *Filobasidiella bacillispora*	Fair conjugation, 0–30% basidiospore viability (F_1 progeny; F_2 not determined)	55–63	Aulakh et al. (1981)
Issatchenkia scutulata var. *scutulata* × *Issatchenkia scutulata* var. *exigua*	Good conjugation, ascospores viable ($F_1 = 5\%$; $F_2 = 17\%$)	21–26	Kurtzman et al. (1980b)
Pichia amylophila × *Pichia mississippiensis*	Good conjugation, ascospores not viable	20–27	Kurtzman et al. (1980a)
Hansenula bimundalis × *Hansenula americana*	Poor conjugation, ascospores not produced	21	Kurtzman (1984b)
Pichia opuntiae var. *opuntiae* × *Pichia opuntiae* var. *thermotolerans*	Infrequent conjugation, ascospores produced rarely	28	Phaff and Holzschu (1981), Holzschu (1981)
Pichia opuntiae var. *opuntiae* × *Pichia antillensis*	No conjugation, ascospores not produced	55	Starmer et al. (1984)
Hansenula alni × *Hansenula wingei*	Poor conjugation, ascospores not produced	6–10	Fuson et al. (1979)
Issatchenkia orientalis × *Issatchenkia occidentalis*	Infrequent conjugation, ascospores not produced	3–8	Kurtzman et al. (1980b)

[a] The names used are those appearing in the original publications.

species characterized by traditional means usually show 60–100% DNA complementarity.

Species boundaries can be prescribed on the basis of a number of criteria, but for sexually reproducing species, ability to hybridize and produce fertile successive generations would seem to be of cardinal importance. Dobzhansky (1976) noted that, among sexually reproducing and outbreeding organisms, species can be defined as Mendelian populations or arrays of populations that are reproductively isolated from other population arrays. He cited a number of examples of higher eukaryotes where interspecific hybridizations have been documented (*Drosophila* spp., mammals, frogs, fishes and plants). Fertility varies among hybrids, being rather rare among animal species but much more common among plants. Since conjugation in bacteria is infrequent and may not involve the entire genome, genetic studies could not form the basis for interpretation of DNA complementarity studies among these prokaryotes. Many yeasts, on the other hand, have known sexual states and with such species hybridization offers a means for correlating results from DNA studies with actual biological phenomena.

Comparisons of extent of nDNA complementarity with measurements of fertility among pairs of heterothallic yeasts have shown that increasing DNA relatedness parallels increased fertility, and that strains showing DNA relatedness at 70% or greater are usually conspecific (Table 10). This same trend was noted for homothallic yeasts (Kurtzman 1987b, 1991a). This is not surprising because homothallics often show some outbreeding through conjugation between independent cells. From these comparisons, the following predictions can be made concerning DNA complementarity and biological relatedness among strains: (1) conspecific strains generally exhibit in excess of 70% DNA relatedness; (2) varietal designations can be accorded to those strains showing 40–70% DNA relatedness unless genetic crosses demonstrate the absence of interfertility. *Issatchenkia scutulata* and its variety *exigua* show some intervarietal fertility and at 25% DNA relatedness appear to be an exception. Conversely, *S. cerevisiae* and *S. pastorianus* show 58% relatedness, but appear to be independent species (Vaughan-Martini and Kurtzman 1985); (3) strains showing less than 40% DNA relatedness are nearly always genetically isolated and represent independent species.

Application of the preceding predictions of relatedness to anamorphic species may not be appropriate and can be criticized on the basis that species not undergoing sexual reproduction may evolve at different rates than teleomorphic taxa. A counter argument is that since sexual states are being continually discovered for anamorphic species, perhaps few yeasts are actually devoid of a meiotic cycle. Furthermore, the frequency of sexual reproduction for teleomorphic species in their natural habitats is unknown and many may be functional anamorphs. Perhaps the most definitive observation at present is that there appears to be neither a greater nor lesser range of DNA relatedness among strains of anamorphic species than found among teleomorphic species. As a consequence, application of the preceding predictions for recognition of anamorphic species from comparisons of DNA relatedness does not appear unreasonable.

7. Impact of DNA reassociation studies on yeast systematics

The information provided by DNA reassociation studies has had a profound impact on yeast systematics. Most notably, it has shown that many morphological and physiological characters used for definition of species and genera are taxon-specific and without phylogenetic significance. These characters include fermentation of glucose, nitrate assimilation, and presence or absence of pseudo- and true hyphae (Table 11). DNA reassociation

Table 11
Nuclear DNA relatedness between yeast species differing in traditional taxonomic characteristics

Species[a]	Characteristic	+ or −	DNA relatedness (%)	Reference
Candida slooffii	Pseudohyphae	+	80	Mendonça-Hagler and Phaff (1975)
Torulopsis pintolopesii		−		
Hansenula wingei	True hyphae	+	78	Fuson et al. (1979)
H. canadensis		−		
Debaryomyces formicarius	Glucose ferm.	+	96	Price et al. (1978)
D. vanriji		−		
Schwanniomyces castellii	Lactose assim.	+	97	Price et al. (1978)
S. occidentalis		−		
Hansenula minuta	Nitrate assim.	+	75	Kurtzman (1984a)
Pichia lindneri		−		
Sterigmatomyces halophilus	Nitrate assim.	+	100	Kurtzman (1990a)
S. indicus		−		

[a] The names used are those appearing in the original publications.

Table 12
Extent of nDNA reassociation between *Saccharomyces cerevisiae* and other species of *Saccharomyces* sensu stricto[a]

Species	DNA rel. (%)	Species	DNA rel. (%)
S. aceti	96	*S. hispalensis*	94
S. beticus	99	*S. italicus*	96
S. capensis	94	*S. norbensis*	90
S. chevalieri	96	*S. oleaceus*	94
S. cordubensis	100	*S. oleaginosus*	89
S. coreanus	94	*S. prostoserdovii*	93
S. diastaticus	92	*S. bayanus*	5
S. gaditensis	95	*Debaryomyces melissophilus*	6
S. hienipiensis	95		

[a] From Vaughan-Martini and Kurtzman (1985).

has also demonstrated the synonymy of quite a large number of species. This is illustrated by Table 12 where it is shown that 15 *Saccharomyces* "species" are conspecific with *S. cerevisiae.* Numerous other equally important examples of the impact of DNA reassociation are found in the *Comments* sections of species and genus descriptions later in this book.

Chapter 10

Ribosomal RNA/DNA sequence comparisons for assessing phylogenetic relationships

C.P. Kurtzman and P.A. Blanz

Contents

1. Introduction

Comparisons of ribosomal RNA (rRNA) and its template ribosomal DNA (rDNA) have been used extensively in recent years to assess both close and distant relationships among many kinds of organisms. The interest in rRNA/rDNA comes from two important properties: (1) ribosomes are present in all cellular organisms and appear to share a common evolutionary origin, thus providing a molecular history shared by all organisms, (2) some rRNA/rDNA sequences are sufficiently conserved that they are homologous for all organisms and serve as reference points that enable alignment of the less conserved areas used to measure evolutionary relationships.

In this review, we will describe the impact that rRNA/rDNA comparisons are beginning to have on our understanding of evolutionary relationships among the yeasts and how this knowledge will influence development of a system of classification based on phylogeny. Additionally, we will discuss application of this new information to the practical goal of rapid yeast identification.

2. Methods for isolation and characterization of rRNAs and rDNAs

rRNAs occur in several size classes in eukaryotes; the genes coding for large (25S to 28S), small (18S) and 5.8S rRNAs occur as tandem repeats with as many as 100 to 200 copies. The separately transcribed 5S rRNA gene may also be included in the repeat (Garber et al. 1988). Each of the rRNA size classes has been examined for the extent of phylogenetic information present.

The large quantities of rRNAs expressed by cells make isolation and purification of these molecules relatively easy despite the nearly ubiquitous occurrence of quite stable RNases. Numerous methods for isolation and purification have been described. The procedures of Chirgwin et al. (1979) and their modification by Kurtzman and Liu (1990) are generally satisfactory. Techniques for the isolation and characterization of rDNA have been described by White et al. (1990) and Vilgalys and Hester (1990).

Methods for sequencing nucleic acids are now commonplace. Techniques for 5S rRNA sequencing have been summarized and discussed by Walker (1984, 1985a). The procedures used for sequencing large and small subunit RNAs and DNAs are based on the dideoxy method of Sanger et al. (1977). Lane et al. (1985) have described the application of this method to rRNA sequencing through use of oligonucleotide primers and reverse transcriptase. Most initial comparisons of yeasts and other microorganisms were based on reverse transcriptase mediated sequencing of rRNAs because of the relative simplicity of this method over earlier rDNA sequencing techniques. Complete sequences often were not determined because McCarroll et al. (1983) and Lane et al. (1985) demonstrated that partial sequences of small subunit rRNAs provided essentially the same phylogenies as complete sequences.

White et al. (1990) and Kaltenboeck et al. (1992) provided protocols for sequencing rDNA using specific oligonucleotide primers and the polymerase chain reaction (PCR). rDNA sequencing results in fewer artifacts than rRNA sequencing, and the method offers the opportunity to sequence both strands of the rDNA genes, thus further reducing errors. Recently, methodologies again evolved with the introduction of automated sequencers.

3. Estimates of relatedness from rRNA/rDNA comparisons

3.1. rRNA/rDNA reassociation

The first extensive use of rRNA comparisons for yeast systematics was described by Bicknell and Douglas (1970), who measured species divergence from extent of reassociation between tritium-labeled 25S rRNA and complementary sites on filter-bound nuclear DNA. This and similar methods have been used by other workers, but because all species pairs must be tested, the comparison of large numbers of taxa is quite laborious. Another aspect of this procedure is that as evolutionary distances increase, a point is reached at which there is insufficient base sequence similarity to allow duplexing of paired molecules. It has been suggested that sequences must

exhibit 75–80% or greater similarity before reassociation can occur (Bonner et al. 1973, Britten et al. 1974).

3.2. Restriction fragment length polymorphisms of rDNA

Because rDNAs occur in multiple copies, they lend themselves to analysis based on restriction fragment length polymorphisms (RFLPs). Magee et al. (1987) treated rDNAs from several medically important *Candida* species with a variety of restriction endonucleases and concluded that *C. guilliermondii*, *C. tropicalis* and *C. albicans* produced sufficiently different digestion patterns to allow recognition of each species. Similar results were obtained by Vilgalys and Hester (1990) for several species of the genus *Cryptococcus*. Lachance (1990a) used RFLP patterns to map the genetic profiles of 125 isolates of the cactus yeast *Clavispora opuntiae* that had been collected worldwide. Nearly all restriction sites that allowed discrimination of individual strains were located in the hypervariable intergenic spacer region.

Data from the preceding studies show that RFLP patterns allow recognition of individual species as well as individual strains of a species. Consequently, the method has considerable diagnostic value. Estimates of evolutionary relationships from RFLP patterns have been reported for species assigned to *Candida* (Magee et al. 1987) and to *Cryptococcus* (Vilgalys and Hester 1990). Such estimates would be expected to be less accurate than estimates derived from sequence comparisons because as evolutionary distances increase, the extent of pattern similarities becomes less certain.

3.3. 5S rRNA

Because of the conserved nature and small size (ca. 120 nucleotides) of 5S rRNAs, their sequences are easily determined and have been widely used for estimating broad phylogenetic relationships (Hori and Osawa 1979). Walker and Doolittle (1982) compared 5S rRNAs from eight basidiomycetes, including four yeasts, and concluded that sequence similarity correlated with the structure of hyphal septa (i.e., simple pores versus dolipores). The report of Gottschalk and Blanz (1984) that rust fungi, which have simple septal pores, cluster with the group defined by Walker and Doolittle as having dolipore septa proved incorrect because contaminating yeasts had been sequenced instead of the yeast stages of the rusts (P.A. Blanz, unpublished data).

Based on 5S rRNA data, organisms of unknown systematic position can be grouped with known taxa. 5S rRNA nucleotide sequences allowed placement of *Microstroma juglandis* among the basidiomycetes (Gottschalk and Blanz 1984) whereas *Kabatiella microstricta* was shown to be ascomycetous (Gottschalk and Blanz 1985). *Agaricostilbum pulcherrimum* was the most divergent of all basidiomycetes examined (Blanz and Unseld 1987), while *Septobasidium carestianum* is related to *Phleogena faginea* (Blanz and Gottschalk 1986) and the rusts (Müller 1989). On the basis of 5S rRNA sequences, the smut fungi were found to form two distinct groups. One parasitizes dicotyledonous host plants while the other parasitizes grasses (Blanz and Gottschalk 1984). The profound genetic divergence between these two groups of smuts is consistent with differences in morphological and chemical characters (G. Deml, personal communication). The affiliation of *Graphiola phoenicis* with the graminicolous smuts was shown from its 5S rRNA nucleotide sequence (Blanz and Gottschalk 1986). The limited similarity of the 5S rRNAs of *Rhodosporidium malvinellum* and *Rhodosporidium toruloides* (Gottschalk and Blanz 1985) was confirmed by Yamada et al. (1989a) from 18S and 26S rRNA partial nucleotide sequences. From these results, they transferred *R. malvinellum* to the new genus *Kondoa* (Yamada et al. 1989a).

Among ascomycetous yeasts, Komiya et al. (1981) determined the 5S sequence of *Schizosaccharomyces pombe*, and Mao et al. (1982) noted that it differed sufficiently from that of *Saccharomyces cerevisiae* to suggest that these two organisms are phylogenetically quite divergent. Similar results were obtained by Walker (1985a) who further showed the ascomycetes to be divided among three groups: (1) *Schizosaccharomyces* and *Protomyces*, (2) budding yeasts, and (3) filamentous fungi. Despite the importance of these phylogenetic studies, the 5S rRNA molecule is losing its prominence and is being replaced by the informationally richer 18S and 26S rRNA molecules which can now be easily sequenced.

3.4. 5.8S rRNA

With about 160 nucleotides, the 5.8S rRNA molecule is not much larger than 5S rRNA. Unlike the latter molecule, 5.8S rRNA contains modified nucleotides as do the 18S and 26S rRNA molecules. Consequently, when enzymatic sequencing is used as was done for 5S rRNA, the modified nucleotides are sometimes difficult to determine. Despite these disadvantages, several 5.8S nucleotide sequences have been analyzed, but only a few phylogenetic comparisons have been deduced from these data (Walker 1985b). More 5.8S rDNA data will become available as the ITS regions of the rDNA tandem repeats are analyzed because the 5.8S rRNA gene is located between the ITS regions.

3.5. Small subunit and large subunit rRNA/rDNA sequences

3.5.1. Close relationships: The compilation of large subunit RNA sequences by Gutell and Fox (1988) demonstrated the 5′ end of this molecule to be quite variable and of potential use for detection of closely related species. Peterson and Kurtzman (1991) examined sequence divergence in this region for sibling species pairs from several yeast genera. These data showed that nucleotide differences in region 25S–635 (domain D2, Guadet et al. 1989) are sufficient to separate nearly all

Table 13
Extent of ribosomal RNA sequence divergence among sibling yeast species[a]

Species pair	Percent nDNA relatedness[b]	Percent nucleotide differences in region 25S–635[c]
Heterothallic species		
Saccharomyces cerevisiae × *S. pastorianus*	58	5.4
Saccharomyces cerevisiae × *S. bayanus*	10	5.4
Saccharomyces pastorianus × *S. bayanus*	70	0.0
Pichia mississippiensis × *P. amylophila*	25	2.0
Pichia bimundalis × *P. americana*	21	1.0
Issatchenkia scutulata × var. *exigua*	25	5.1
Homothallic species		
Debaryomyces melissophilus × *D.* sp. n.	30	0.7
Saturnispora saitoi × *S. ahearnii*	30	2.0
Williopsis saturnus × var. *sargentensis*	43	0.0

[a] Data from Kurtzman (1984b, 1987b), Kurtzman and Robnett (1991), Kurtzman et al. (1980a,b), Liu and Kurtzman (1991), Peterson and Kurtzman (1990, 1991), Vaughan-Martini and Kurtzman (1985).
[b] Conspecific strains generally exhibit in excess of 70% nuclear DNA relatedness. Varietal designations have been accorded those strains showing 40–70% nDNA relatedness unless genetic comparisons demonstrate the absence of interfertility. *I. scutulata* and its variety *exigua* show some intervarietal fertility and, at 25% DNA relatedness, appear to be exceptional because limited fertility would not be expected.
[c] Ca. 300 nucleotides in region 25S–635 (= region D2).

sibling species (Table 13). One exception is the pair *Saccharomyces bayanus/S. pastorianus*. It is believed that the latter species arose as a partial amphidiploid following chance hybridization between *S. cerevisiae* and *S. bayanus*, and that it retains the rDNA of *S. bayanus* (Kurtzman and Robnett 1991, Peterson and Kurtzman 1991, Vaughan-Martini and Kurtzman 1985). *Williopsis saturnus* and its variety *sargentensis*, which show no nucleotide differences in the region sequenced, are insufficiently studied to comment on their apparent lack of divergence (Liu and Kurtzman 1991). Some sibling species pairs show a five-fold difference in substitutions over that of other pairs, but this may not be definitive evidence for proposing unequal rates of nucleotide substitutions among species until the genetic processes that initiate species formation are better understood. With few exceptions, the D2 region is sufficiently variable to recognize ascomycetous and basidiomycetous yeast species, including most sibling pairs. Conspecific strains ordinarily show 0–1% divergence in this region, whereas the distantly related species *Pichia bimundalis* and *Schizosaccharomyces japonicus* var. *versatilis* exhibit ca. 47% substitutions (Peterson and Kurtzman 1991).

3.5.2. Distant relationships:

3.5.2.1. Ascomycetous yeasts: The phylogeny of the ascosporogenous yeasts has been vigorously debated since the time of Guilliermond (1912) and before. Some have viewed the yeasts as primitive fungi while others perceived them to be reduced forms of more evolved taxa. Cain (1972) has been a proponent of this latter idea, arguing that hat- (galeate)-spored genera such as *Pichia* and *Cephaloascus* are likely to be reduced forms of the perithecial euascomycete genus *Ceratocystis*. Redhead and Malloch (1977) and von Arx and van der Walt (1987) accepted this argument and commingled yeasts and mycelial taxa in their treatments of the Endomycetales and Ophiostomatales.

Examination of rRNA/rDNA sequence divergence from a limited number of taxa indicated the ascosporogenous yeasts, with the exception of *Schizosaccharomyces*, to form a monophyletic group (clade) distinct from the filamentous species (Barns et al. 1991, Bruns et al. 1991, Hausner et al. 1992, Hendriks et al. 1992, Kurtzman 1993a, 1994, Nishida and Sugiyama 1993, Walker 1985a, Wilmotte et al. 1993). Kurtzman and Robnett (1994a) analyzed rRNA sequence divergence from type species of all cultivatable ascomycetous yeasts and yeastlike taxa. This work demonstrated the yeasts, as well as yeastlike genera such as *Ascoidea* and *Cephaloascus* to comprise a clade sister to the "filamentous" ascomycetes (euascomycetes). *Schizosaccharomyces, Taphrina, Protomyces* and *Saitoella* form a divergent clade basal to the yeast–euascomycete branch. *Eremascus*, which forms asci unenclosed in a fruiting body, aligned with the euascomycete clade. These results substantiate the long-held observation that yeasts cannot be recognized solely on the basis of presence or absence of budding. Such members of the yeast clade as *Ascoidea, Eremothecium* (and its synonym *Ashbya*) show no typical budding, whereas *Aureobasidium, Phialophora* and certain other genera of euascomycetes are usually dimorphic. Budding is also a common mode of vegetative reproduction among many basidiomycetous genera. Similarly, vegetative reproduction by fission is shared by *Dipodascus* and *Galactomyces*, members of the yeast clade, as well as by the distantly related genus

Schizosaccharomyces. Sexual states of all members of the yeast clade are characterized by asci unenclosed in a fruiting body. This feature is shared by only a few taxa outside the yeast clade such as *Eremascus* and *Schizosaccharomyces*. *Myriogonium* and *Trichomonascus* form unenclosed asci but may be euascomycetes.

The impact of rRNA/rDNA comparisons on the taxonomy of ascomycetous yeasts is just beginning. Additional work is required to evaluate the assignment of species to genera and the relationships among genera. However, major findings to date include: (1) yeasts and yeastlike species are phylogenetically separate from the euascomycetes, (2) the fission yeast genus *Schizosaccharomyces* is phylogenetically distant from the "budding" yeast clade and from the euascomycetes, resulting in the reassignment of the fission yeasts to a separate order, the *Schizosaccharomycetales* (Eriksson et al. 1993, Kurtzman 1993a) and, (3) the demonstration that many phenotypic characters such as ascospore morphology are poor indicators of phylogeny (Kurtzman and Robnett 1991, 1994b, Liu and Kurtzman 1991). In a number of cases, these findings have been used to redefine taxa, and additional details may be found in the treatments of *Debaryomyces, Eremothecium, Lipomyces, Pichia, Saturnispora* and *Williopsis*. On the basis of rDNA sequence comparisons, Nishida and Sugiyama (1993) suggested the name Archiascomycetes for the clade comprising *Schizosaccharomyces, Saitoella, Taphrina, Protomyces* and *Pneumocystis*.

3.5.2.2. Basidiomycetous yeasts: Two morphologically distinct teleomorph states are found among the basidiomycetous yeasts (Fell and Kreger-van Rij 1984, Boekhout et al. 1993). In the first, teliospores are formed and germinate to produce a basidium (metabasidium) that bears basidiospores. This type of sexual cycle shows considerable similarity to the rust and smut fungi. The second type of sexual state lacks teliospores. Basidia develop on hyphae or yeast cells and give rise to basidiospores in a manner similar to the Tremellales (jelly fungi). Additionally, taxa may be defined from presence or absence of carotenoids, ballistoconidia, type of hyphal septum (simple or the ultrastructurally more complex dolipore), and cellular xylose, which evidently arises from extracellular polysaccharides (Golubev 1991a).

Guého et al. (1989) presented an overview of phylogeny of basidiomycetous yeasts from measurements of divergence among partial sequences of large and small subunit rRNAs. Three major groups were resolved: (1) teliospore formers with hyphae having simple septal pores, (2) teliospore formers with hyphae having dolipore septa and, (3) non-teliospore formers with hyphae having dolipore septa. Relative to other taxonomic groups, the basidiomycetous yeasts appear to be a sister group to the Agaricales (Berbee and Taylor 1993).

At present, the most extensive phylogenetic comparison of basidiomycetous yeasts is that by Fell et al. (1992) who examined 117 species assigned to 23 genera. A 247-nucleotide segment in the D2 region was sequenced. Although this region resolves closely related species, it has too few phylogenetically informative sites to accurately assess more distant relationships. Nonetheless, the analysis generally supports the concept that taxa assigned to the Tremellales are characterized by dolipore septa and cellular xylose, whereas taxa placed in the Ustilaginales form teliospores, have simple septal pores, and lack cellular xylose. Some exceptions were noted. The teleomorphic genus *Erythrobasidium* does not form teliospores as do other members of the clade, but *Cystofilobasidium* and *Mrakia*, both members of the Tremellales, do form teliospores.

Ballistoconidia and carotenoids are found among many genera of the basidiomycetous yeasts, suggesting these traits to be ancestral, but not always expressed, thus rendering them of little value for defining taxa. These conclusions were also drawn by Nakase et al. (1993). Heterogeneity of coenzyme Q composition occurs in many currently defined genera and will require additional study before its taxonomic significance is fully understood. rRNA/rDNA sequence analyses demonstrated *Rhodotorula, Sporobolomyces, Cryptococcus,* and *Bensingtonia* to be polyphyletic, further confirming that commonly used phenotypic characters are insufficient for defining anamorphic genera. Additional comparisons of basidiomycetous taxa can be found in the studies by Blanz et al. (1989), De Wachter et al. (1992), Fell and Kurtzman (1990), Guého et al. (1990), Laaser et al. (1989), Nakase et al. (1991a), Sugiyama and Suh (1993), Suh and Sugiyama (1993b), Van de Peer et al. (1992), Yamada and Kawasaki (1989a), Yamada and Nakagawa (1990), and Yamada et al. (1989a,b, 1990a,b).

A strong start has been made to understand the phylogeny of basidiomycetous yeasts from rRNA/rDNA sequence comparisons, but additional sequencing must be done to better resolve taxa. This will include sequences of greater length as well as inclusion of all known species of a group. For example, the anamorphic genera *Tsuchiyaea* and *Ballistosporomyces*, which were recently defined from differences in partial sequences, may overlap with some earlier described genera (Fell et al. 1992).

4. Reliability of rRNA gene trees to infer phylogeny

Calculations of phylogenetic distances from changes in the sequences of macromolecules require that substitutions occur at a predictable rate and preferably one that is constant as well. Ochman and Wilson (1987) reported that nucleotide substitutions in small (16–18S) subunit rRNAs from both prokaryotes and eukaryotes proceed at the same rate (1%/50 million years). Other workers, however, have presented evidence that rates of nucleotide substitution differ among phylogenetic groups and possibly among species within groups (Britten 1986, Field et al. 1988,

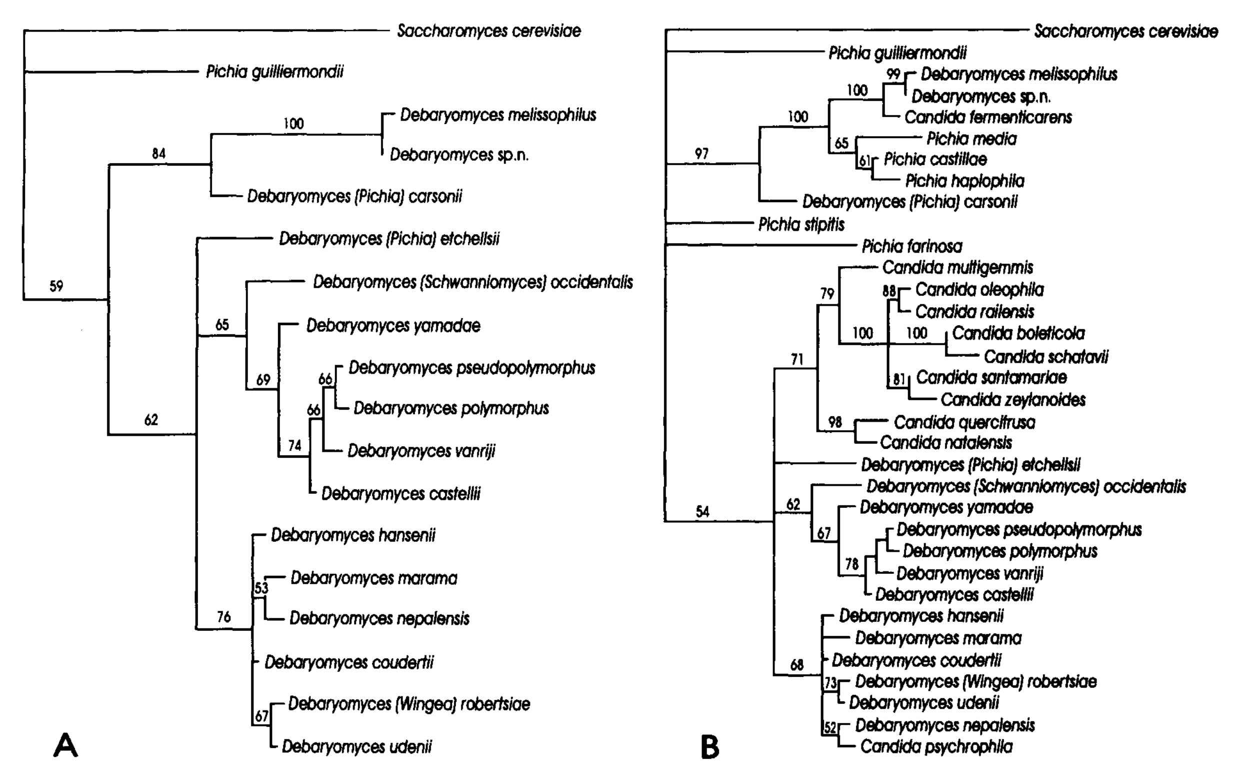


Fig. 17. Phylogenetic analyses of relationships among species of *Debaryomyces* and reference taxa. The trees are derived from maximum parsimony (PAUP) analysis of ca. 600 nucleotides from the 5′ end of large subunit rDNAs. Branch lengths are proportional to nucleotide differences, and the numbers given on branches are the percentage of frequencies with which a given branch appeared in 100 bootstrap replications. Values less than 50% are not given. (A) Phylogenetic tree comprised of described *Debaryomyces* species and two reference taxa. (B) Phylogenetic tree as in A, but with eighteen reference taxa. *D. carsonii, D. melissophilus* and *Debaryomyces* sp. n. are separated from other members of *Debaryomyces* in this analysis. (C.P. Kurtzman and C.J. Robnett, unpublished data).

Weisburg et al. 1989). Methods for calculating evolutionary relationships based on macromolecular changes have been described (Avise 1989, Felsenstein 1988, Holmquist et al. 1988, Lake 1987, Saitou and Nei 1987, Woese 1987), and some of the algorithms are designed to compensate for disproportional rates of substitution, but there still appears to be uncertainty concerning their reliability (Felsenstein 1988). Evolutionary relationships among the bacteria, for example, are still subject to debate (Achenbach-Richter et al. 1988, Gouy and Li 1989, Lake 1987).

There are presently only a few comparisons among the yeasts that examine congruence between phylogenies from rRNA gene sequences and those determined from other molecules. Sequence analysis of orotidine 5′-monophosphate decarboxylase by Radford (1993) demonstrated the budding yeasts and the euascomycetes to be sister groups as shown from rRNA/rDNA sequences. One unusual aspect of this work was placement of the Mucorales as a sister group to the basidiomycetes. Tsai et al. (1994) showed phylogenetic relationships among species of *Epichloë* (Clavicipitaceae) were the same when analyzed from either rDNA sequences or those from the β-tubulin gene. Relationships among species of *Dekkera* and its anamorph *Brettanomyces* were essentially identical when analyzed from either nuclear rDNA sequences or from sequences of the mitochondrially encoded cytochrome oxidase subunit II gene (Boekhout et al. 1994).

Another aspect of gene tree reliability is the method used for its construction. Most investigators now analyze data using phylogeny inference programs based on cladistic principles. These programs often include a statistics package to test the robustness of competing phylogenetic trees. Several recent reviews address these important issues (Avise 1989, Felsenstein 1988, Hillis et al. 1994, Saitou and Imanishi 1989).

Although evidence is strong that molecular comparisons give an accurate approximation of the evolutionary history of an organism, use of phylogenetic trees for definition of taxonomic hierarchies should be done cautiously. One impediment to interpretation of these data is the issue of missing taxa. Taxa may be excluded either because they are extinct or they have not been recognized in nature. An indication of missing taxa is the presence of long branches on phylogenetic trees, assuming rates of nucleotide substitutions are reasonably constant for all species compared.

The taxonomy of *Debaryomyces* is an example of the problems encountered when there is incomplete sampling of species. On the basis of nucleotide similarities, Kurtzman and Robnett (1991, 1994b) proposed transfer of *Schwanniomyces occidentalis* and *Wingea robertsiae*

to *Debaryomyces,* and Yamada et al. (1992d) proposed transfer of *Pichia carsonii* and *P. etchellsii* to this genus. A phylogenetic analysis that includes the preceding four species with previously described *Debaryomyces* species suggests that *Debaryomyces* is monophyletic (Fig. 17A). When additional species are included in the analysis, *D. carsonii, D. melissophilus* and *Debaryomyces* sp. n. are no longer closely allied with other species in the genus (Fig. 17B). If the circumscription of genera is to be based on monophyly of their assigned species, the preceding three species and other members of their clade represent a new genus. Before such taxonomic changes are made, all known yeasts need to be sequenced and compared, taking into account taxa located on the long branches of phylogenetic trees and their possible effect on genus circumscription.

5. Rapid molecular methods for yeast identification

The specificity of nucleic acid sequences has prompted development of several methods for rapid species identification. Because these techniques can detect single nucleotide changes, they should first be tested on a large variety of genetically defined strains to understand species variation. Aberrant strains may represent different species.

The RFLP technique was discussed earlier in the review and has been used extensively in some laboratories. Bruns et al. (1991) listed some of the factors that require attention when using RFLPs. Random amplified polymorphic DNA (RAPD) is another methodology that is becoming widely used. The technique is based on amplification of genomic DNA in the presence of one or more short (ca. 10–15-mers) oligonucleotide primers of random sequence. The amplified products are visualized on an agarose gel and strains identified from matching band patterns. Hadrys et al. (1992) discussed details of this procedure noting points of technical difficulty. Poor reproducibility of band patterns may occur, and apparently results from small differences in reagent concentrations and from small variations in thermal cycler temperature control.

Fell (1993) applied a three-primer, PCR-based technique to yeast identification that appears species-specific. The reaction mix includes genomic DNA, two external primers for the D1/D2 region of large subunit rDNA, and a species-specific internal primer. The external primers allow amplification of the ca. 600-nucleotide D1/D2 region, but in the presence of a species-specific primer (third primer), the amplification product is shorter and easily detected on agarose gel. Other molecular technologies for identification include development of species-specific oligonucleotide probes and the sequencing of species-specific regions of DNA. In this latter concept, it is practical to rapidly identify taxa from their sequences in the 600-nucleotide D1/D2 region because many laboratories are beginning to use automated sequencers.

Part IV

Methods

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Chapter 11

Methods for the isolation, maintenance and identification of yeasts

D. Yarrow

Contents

1. Isolation of yeasts

Yeasts have been recovered from widely differing aquatic and terrestrial sources, as well as from the atmosphere. Many yeasts occur widely, whereas some appear to be confined to restricted habitats. Yeasts seldom occur in the absence of either molds or bacteria. Consequently, selective techniques are often used for recovery of yeasts, employing media which permit the yeast to grow while suppressing molds and bacteria. The composition of such media is determined by the fact that yeasts are, as a rule, capable of developing at pH levels and water activities which reduce or inhibit the growth of bacteria. Antibiotics may also be used to suppress bacteria. Fungistatic agents for the suppression of molds should be used with caution because such compounds may also inhibit some yeasts.

Cultures are usually incubated at 20–25°C because most yeasts are mesophilic; however, temperatures between 4 and 15°C are essential for psychrophilic taxa. Higher temperatures, in the range of 30–37°C, are often required for yeasts that are strictly associated with warm-blooded sources.

Certain yeasts, such as species of the genera *Cyniclomyces* and *Malassezia*, have exceptional nutritional requirements. The reader should consult the chapters dealing with these taxa for details. The medium formulated by Leeming and Notman (1987) allows species of *Malassezia* to grow well at incubation temperatures between 30 and 35°C.

Anyone interested in the recovery of yeasts from natural substrates is also referred to the informative publications of Beech and Davenport (1969, 1971) and Davenport (1980b), which deal with the isolation of non-pathogenic yeasts. The publications of Buckley (1971) and Staib et al. (1989) may be consulted for methods of isolating yeasts from clinical specimens.

1.1. Use of acidic media (pH 3.5–5.0)

Either hydrochloric acid or phosphoric acid is preferred for acidifying media. The use of organic acids, such as acetic acid, is not recommended for general isolation purposes. Such acids are only slightly dissociated at pH 3.5–4.0 and the high concentrations of undissociated acids have an inhibitory effect on most yeasts. Notable exceptions are *Zygosaccharomyces bailii*, *Z. bisporus*, *Schizosaccharomyces pombe*, and some strains of *Pichia membranifaciens* and similar species.

1.1.1. Solid media for direct isolation: When yeasts are present in high numbers they may be isolated by plating the material, or suspensions of the material, on either acidified media or media containing antibiotics.

Agar in media with a low pH is hydrolysed when autoclaved. Therefore, the sterilized molten agar is cooled to approximately 45°C before a determined volume of acid is added. The medium and acid are rapidly mixed and immediately poured into petri dishes. The addition of approximately 0.7% (v/v) 1 N hydrochloric acid to YM agar and glucose–peptone–yeast extract agar usually gives the desired pH of 3.7 to 3.8.

Although many yeasts can be recovered at pH 3.7, some species, notably those of the genus *Schizosaccharomyces*, are inhibited by very acid media and are best isolated on moderately acidic media with a pH in the range 4.5 to 5.0.

Dilution-plate techniques may be used for quantitative studies.

1.1.2. Liquid media for enrichment purposes: When yeasts are present in low numbers, their isolation may require enrichment using media and conditions which favor the growth of yeasts over other microorganisms. In such cases, material is put into a liquid medium with a pH of 3.7 to 3.8.

The development of molds can be restricted by excluding air from the culture by pouring sterile pharmaceutical paraffin over the surface of the media to form a layer about 1 cm deep. This procedure favors the development of fermentative strains, but may fail to recover aerobic strains.

An alternative, and preferable, procedure is to incubate the flasks of inoculated media on a rotary shaker (Wickerham 1951). Molds are prevented from sporulating and aggregate in pellets which are outgrown by yeasts. The yeasts may be separated from the molds either by allowing the pellets of mold to settle for a few minutes and then streaking the suspension of yeasts on to agar in petri

dishes, or by removing suspended pellets by aseptically filtering through a loose plug of sterile glass wool. Both fermentative and non-fermentative strains are recovered by this technique.

Wickerham (1969b) described a very useful medium, which he refers to as IM, for isolating yeasts from soil and insect frass. This medium contains Yeast Nitrogen Base plus glucose and six other carbon sources. The pH is not adjusted and drops after inoculation owing to the removal of the ammonium sulfate which is used as a source of nitrogen by the growing organisms.

1.2. Use of media with high concentrations of sugar

Most yeasts can grow on media with concentrations of sugar that are high enough to inhibit the development of many bacteria.

1.2.1. Solid media for direct isolation: A medium such as glucose–peptone–yeast extract agar or YM agar containing glucose at a concentration of 30 to 50% is suitable for recovering osmophilic and osmotolerant yeasts from foodstuffs and juice concentrates of low water activity. The selective action of such media can be enhanced by lowering the pH to around 4.5. Osmotolerant yeasts recovered in this way can usually be successfully subcultured on media containing successively decreasing amounts of sugar, for example 30, 10, 4 and 2%.

1.2.2. Liquid media for enriching cultures: Yeasts may also be isolated by cultivation in liquid media such as glucose–peptone–yeast extract broth and YM broth containing from 30 to 50% glucose. Osmotolerant molds are not inhibited at these sugar concentrations, therefore incubation on a rotary shaker is recommended. Wickerham (1969b) described another useful medium for the isolation of yeasts from soil and insect frass. This medium, which he refers to as D-20, contains Difco Yeast Nitrogen Base, 20% glucose, 0.1% yeast extract and 0.1% malt extract.

1.3. Use of antibiotics and other inhibitory or selective compounds

Several media containing antibiotics have been described for the isolation of yeasts (Davenport 1980b) and these can be employed as a last resort when other means have failed. Such media may have been compounded for the isolation of a particular genus, species, or of yeasts with a particular property. These techniques rely on the use of antibiotics and either other inhibitors or selective carbon and nitrogen sources. Van der Walt and van Kerken (1961a) described isolating species of *Dekkera* using media containing cycloheximide and sorbic acid at pH 4.8. Van Dijken and Harder (1974) used a medium containing methanol as the sole carbon source, plus cycloserine and penicillin G to inhibit the growth of bacteria, for the isolation of yeasts able to utilize methanol. Kwon-Chung et al. (1978) describe a medium for the isolation of *Filobasidiella neoformans* containing creatinine as nitrogen source and diphenyl to reduce the growth of molds. Another medium for the selective isolation of this species contains Niger seed (*Guizotia abyssinica*), which gives pigmented colonies, and penicillin, streptomycin and gentamicin for the suppression of bacteria (Staib et al. 1989). Beech et al. (1980) reviewed the use of antibiotics such as cycloheximide, aureomycin, chloramphenicol, and penicillin in media for the isolation of yeasts.

1.4. Use of membrane filters

Yeasts may be recovered from liquid substrates (and from solid substrates by first washing them to suspend the yeast cells) by passing the liquid through membrane filters (Mulvany 1969) and then incubating the filters on the surface of a selective agar medium. This technique is particularly useful for recovering yeasts when they are present in very low concentrations.

1.5. Purification of yeast cultures

Isolates are obtained in pure culture from enriched cultures by streaking on a suitable medium, such as glucose–peptone–yeast extract agar and YM agar. Persistent bacterial contamination can be eliminated by acidifying the media or by adding antibiotics. These primary plates are inspected, preferably under low magnification, for the presence of colonies of more than one form. Single, well separated colonies of each form are selected and streaked again. Twice is generally sufficient to obtain pure cultures, but sometimes it may be necessary to streak colonies several times. It must be borne in mind, however, that where two or more forms persistently appear after the replating of a single colony, they may be morphological or sexual variants of a single yeast. In such cases it is necessary to examine the different forms in detail and take into account the possibility that they represent mating types.

2. Maintenance of yeast cultures

Yeast cultures are best maintained on a medium which contains glucose as the only source of carbon as this reduces the risk of changes in growth and fermentative patterns due to the selection of mutants (Scheda 1966). The properties of a strain can change within a few days due to such selection when an unstable strain is cultivated on media which contain malt extract, such as malt agar and YM agar. Many basidiomycetous yeasts do not survive well during prolonged storage on a glucose–peptone medium, although they grow well on it. Potato-dextrose agar is suitable when cultures of such yeasts are to be kept for a long time. The majority of yeasts may be stored at temperatures between 4 and 12°C and subcultured at intervals of 6 to 8 months. Some yeasts, for instance *Arxiozyma* and *Malassezia*, need to be subcultured every month. Strains of species of *Dekkera* and *Brettanomyces* produce excessive amounts of acetic acid; for these it is

best to add 1 or 2% calcium carbonate to the medium to neutralize the acid. Nevertheless, these yeasts still need to be subcultured every two months.

Some ascogenous and basidiosporous yeasts lose their ability to reproduce sexually when maintained by serial cultivation on laboratory media. Some isolates still sporulate well after 50 or more years in cultivation. However, for many strains, ability to sporulate is either impaired or lost within a period that varies from a few weeks to several years. Because of this, it is best to preserve important strains, such as nomenclatural types and reference strains, with one of the more permanent conservation techniques as soon as possible after acquisition. Suitable techniques are: lyophilization (see Kirsop and Kurtzman 1988), L-drying (Mikata and Banno 1989), and freezing in either liquid nitrogen or a mechanical freezer at temperatures between −60° and −135°C. Freezing is the preferred method at the Centraalbureau voor Schimmelcultures at present; cultures of all strains held have been frozen and recovered from liquid nitrogen and many have been successfully kept at −75°C. The preparation of cultures for freezing is simple and quick. The general method as used at the Centraalbureau voor Schimmelcultures is as follows: short lengths of polypropylene drinking straws are sealed at one end, labelled with a black felt-tipped pen (Pentel Permanent Marker), and sterilized in the autoclave at 121°C for 15 min. The strain to be frozen is grown for about 24 h in 3.0 ml of liquid medium on a shaker before adding 1.0 ml of a 60% solution of glycerol in water. An amount of the resulting suspension is pipetted into the straws sufficient to half fill them. The straws are then closed by clamping the open ends in the jaws of a sealing machine for plastic packages. The cultures are then either frozen at approximately −30°C for between 30 and 60 min before being placed in the storage tank under liquid nitrogen, or put directly into a freezer cabinet at −75°C. Sterile plastic ampoules suitable for use in liquid nitrogen are commercially available but are more expensive and take up more storage space.

2.1. Media

(1) YM broth (yeast extract–malt extract broth). Dissolve 3 g of yeast extract, 3 g of malt extract, 5 g of peptone, and 10 g of glucose in 1 liter of water. The pH reaction of the medium ranges between 5 and 6 depending on the batch of ingredients. The medium is dispensed into containers and autoclaved at 121°C for 15 min. This medium is commercially available as Bacto YM Broth (Difco).

(2) YM agar (yeast extract–malt extract agar). This medium is prepared by dissolving 20 g of agar in a liter of YM broth. The medium is sterilized by autoclaving at 121°C for 15 min. This medium is commercially available as Bacto YM agar (Difco).

(3) Malt extract (after Lodder and Kreger-van Rij 1952). Mix 1 kg of malt with 2.6 liters of tap water and heat to 45°C for 3 h with continuous stirring. Then raise the temperature to 63°C for 1 h. Next filter the mixture through cheese cloth. The filtrate is then filtered through paper and diluted to a density of 15° Balling using a flotation meter. The pH is adjusted to 5.4, if necessary, and the medium is sterilized by autoclaving at 115°C for 15 min. Commercial products in the form of powder or syrup are available from various suppliers.

(4) Malt agar (malt extract agar). Malt extract is diluted to a density of 10° Balling and 2% (w/v) of agar is added. Malt agar, or wort agar, is available commercially in powder form. The medium is sterilized by autoclaving at 115°C for 15 min.

(5) Glucose–peptone–yeast extract agar (GPY agar). Add 40 g of glucose, 5 g of peptone, 20 g of agar, and 500 ml of yeast infusion to 500 ml of demineralized water and dissolve. Alternatively, 5 g of powdered yeast extract in 500 ml of demineralized water can be substituted for the yeast infusion.

(6) Sabouraud's 4% glucose agar. Dissolve 10 g of peptone and 40 g of glucose in 1 liter of demineralized water, adjust the pH to 7.0 and then add 20 g of agar. Sterilize by autoclaving at 121°C for 15 min. Commercial products in dried powder form are available from various suppliers.

(7) Niger seed agar 1 (Kwon-Chung et al. 1982b). Dissolve 1 g of glucose and 20 g of agar in 800 ml of demineralized water and add 200 ml of Niger seed infusion. Chloramphenicol (40 mg/ml) may be added before sterilizing at 121°C for 15 min, and diphenyl solution (see below) when the medium has been cooled just before pouring into petri dishes.

The Niger seed infusion is prepared by autoclaving 70 g of ground or pulverized seeds of *Guizotia abyssinica* in 350 ml of demineralized water for 10 min at 110°C and filtering the infusion through gauze.

(8) Niger seed agar 2 (Staib et al. 1989). Pulverize 50 g of Niger seed in a blender, boil in 1 liter of demineralized water for 30 min, filter through paper, and restore the final volume to 1 liter. Dissolve 10 g of glucose, 1 g of potassium dihydrogen phosphate, 1 g of creatinine, and 15 g of agar in this solution. Sterilize by autoclaving at 110°C for 20 min. Add streptomycin (40 E/ml), penicillin (20 E/ml), and diphenyl solution (see below) when the medium has cooled to about 50°C. *Filobasidiella (Cryptococcus) neoformans* produces brown, usually mucoid, colonies after 3–8 days at 25°C.

(9) Leeming and Notman agar (LNA) for *Malassezia* species. Dissolve 10 g of bacteriological peptone, 0.1 g of yeast extract, 5 g of glucose, 8 g of desiccated ox bile, 1 ml of glycerol, 0.5 g of glycerol monostearate, 0.5 ml of Tween 60, 10 ml of whole-fat cow's milk, and 12 g of agar in 1 liter of

demineralized water. Sterilize by autoclaving at 110°C for 15 min.

(10) Diphenyl solution. Dissolve 1 g of diphenyl in 100 ml of 95% ethanol. Add 10 ml aseptically to 1 liter of molten medium when it has cooled to approximately 45°C. Diphenyl is used to inhibit the growth of molds.

(11) Yeast infusion. Yeast infusion is prepared by mixing 1 kg of compressed baker's yeast with 5 liters of demineralized water and heating to 50°C for 24 h. Add the whites of 2 eggs to clarify the liquid, shake well and filter through thick paper. Sterilize by autoclaving at 121°C for 15 min. Alternatively, 5 g of powdered yeast extract dissolved in 1 liter of water may be used.

3. Procedures for the identification of yeasts

Workers should always convince themselves beyond any doubt that they are dealing with pure cultures before proceeding to the determination of the morphological, sexual, biochemical, and physiological properties of the isolates.

3.1. Characteristics of vegetative reproduction

3.1.1. Modes of vegetative reproduction: Vegetative or asexual reproduction occurs in yeasts by budding, by fission, and by the production of conidia on short stalks called sterigmata. Observing how the conidia are formed (conidiogenesis) can provide information which aids the identification of a strain.

Buds may arise either on yeast cells or on hyphal cells. Budding is initiated by the formation of a small evagination or outgrowth at some point on the surface of the cell. The cell remains more or less constant in size during subsequent development, while the bud (blastospore or blastoconidium) increases in size to form a new cell which, usually after some time, separates from the parent (mother) cell. Budding is termed holoblastic or enteroblastic, depending on how the bud is formed in terms of the fine structure of the cell wall. All layers of the wall of the parent cell are involved in the formation of a holoblastic bud, and the bud separates, usually on a narrow base; a scar remains through which no further budding occurs. Von Arx and Weijman (1979) consider holoblastic budding characteristic of the Saccharomycetales and their anamorphic states. When budding is enteroblastic, the first bud arises through a rupture in the wall of the parent cell, through which the inner-most layer evaginates and ultimately grows out to form the outer-most layer of the bud. The site of budding is eventually surrounded by a collarette due to the recurrent formation and abscission of a succession of buds arising from the inner layer of the wall of the cell. Enteroblastic budding is characteristic of basidiomycetous yeasts.

Budding is also classed in terms of the position of the site where it occurs. Budding restricted to one pole of the cell is termed monopolar (Fig. 18); budding at both poles of the cell is termed bipolar (Fig. 19). When the buds are abstricted on a rather broad base by the formation of a cross wall, the process is referred to as "budding on a broad base" and "bud fission". Recurrent budding leads to the formation of multiple scars or annellations at the poles of the cell (Streiblová 1971). Bipolar budding is characteristic of the apiculate yeasts. Budding from various sites on the cell is termed multilateral or multipolar (Fig. 20).



Fig. 18. Monopolar budding (from van der Walt and Yarrow 1984a).

Fig. 19. Bipolar budding (from van der Walt and Yarrow 1984a).

Budding is also described in terms of the way successive buds are produced. Sympodial budding is on a conidiophore that extends in growth by a succession of apices. A conidium is produced at each apex and the growth continues to the side of the apex; the result is a zigzag appearance (see *Sympodiomyces*, p. 603). Acropetal budding is the formation of successive buds in a chain with the youngest at the apex. Basipetal budding is the formation of successive buds with the oldest at the apex.

Reproduction by fission is the duplication of a vegetative cell by means of a septum growing inwards from the cell wall to bisect the long axis of the cell. The newly formed fission cells, which are arthroconidia (arthrospores), elongate and the process is repeated. Recurrent fission by a cell may give rise to transverse multiple scars or annellations (Streiblová 1971). This

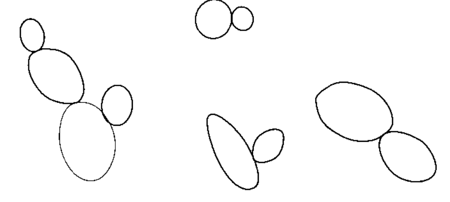


Fig. 20. Multilateral budding (from van der Walt and Yarrow 1984a).

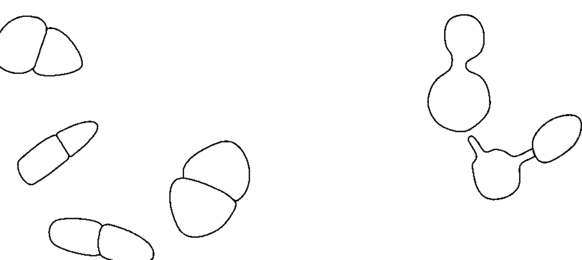


Fig. 21. Fission (from van der Walt and Yarrow 1984a).

Fig. 22. Conidia on stalks (from van der Walt and Yarrow 1984a).

manner of reproduction is characteristic of the genus *Schizosaccharomyces* (Fig. 21).

Reproduction by the formation of conidia borne on stalk-like tubular structures is uncommon among the yeasts. It entails the formation by a cell of one or more tubular protuberances, each of which gives rise to a terminal conidium (Fig. 22). On maturation the conidium is disjointed at a septum either in the mid-region of the tube (e.g., *Sterigmatomyces*) or close to the bud (e.g., *Fellomyces*). The conidia are not forcibly discharged.

Yeasts may also reproduce by the formation of more or less filamentous structures consisting of either pseudohyphae or true hyphae (see section 3.1.2.2) and the formation of forcibly discharged spores or ballistospores (see section 3.1.2.6).

3.1.2. Characteristics of vegetative cells:

3.1.2.1. The morphology of vegetative cells: Cells can be globose, subglobose, ellipsoidal, ovoidal, obovoidal, cylindrical, botuliform, bacilliform, elongate, apiculate, ogival, lunate, or triangular. Definitions and illustrations of the various possibilities can be found in *Ainsworth and Bisby's Dictionary of the Fungi* (Hawksworth et al. 1983, 1995). The shape may reflect the mode of reproduction and, in some cases, it is a characteristic of particular genera or species. Some examples are: the lemon-shaped cells of the apiculate yeasts *Hanseniaspora* and *Wickerhamia*, the bottle-shaped cells of *Malassezia*, the triangular cells of *Trigonopsis*, and the lunate cells of *Metschnikowia lunata* and *Candida peltata*.

Methods

(1) Growth in liquid media. The morphology of cells is examined in cultures grown in liquid media; the most generally used are: glucose–peptone–yeast extract broth, malt extract, and YM broth. Cells from a young actively growing culture are inoculated into 30 ml of medium in a 100-ml, cotton-plugged Erlenmeyer flask and incubated for 2–3 days. Some workers use tubes with a diameter of 16 mm containing 5 ml of medium. Incubation is normally at 25°C, but some strains may require other temperatures. The culture is then examined, noting the shape of the cells, their mode of reproduction, whether they are single, in pairs, or aggregated in large clumps. The length and breadth of at least 20 cells are measured and the extreme values noted.

The characteristics of the culture are noted when the cells are examined and again after 4 weeks, usually at either room temperature or 20°C. The growth of yeasts in liquid media can result in the formation of a compact, coherent, flocculent or mucoid sediment, a ring, floating islets or a pellicle. The pellicle, if formed, can be dry, moist, dull, glistening, smooth, wrinkled, or creeping up the sides of the flask. It is either formed rapidly and is present at the first inspection or it is not present until the second inspection and, even then, it may not completely cover the surface of the medium. Should the yeast form true hyphae copiously, these may produce a thick tenacious growth. This sometimes results in the contents of the flask or tube becoming a mucoid mass. Liquid media are unsuitable for cultivating some yeasts, notably *Malassezia* and *Oosporidium* species.

(2) Growth on solid media. Solid media are sometimes used as well as, or as an alternative to, liquid media for the examination of the morphology of cells. The most commonly used media are glucose–peptone–yeast extract, malt, morphology, and YM agars. A slant or, if morphology agar is used, either a Dalmau plate or an agar-covered slide, is inoculated with cells from a young actively growing culture. (See the next section for the preparation of Dalmau plates and slides.) The slants, plates or slides are incubated for 1–7 days and then the characteristics of the culture are noted. The cell morphology, as described in the previous section, is also examined in some laboratories.

The following features of the appearance of cultures are recorded:

Texture: whether mucoid, fluid or viscous, butyrous, friable, or membranous. Mucoid growth is frequently associated with encapsulation of cells as a result of the production of extracellular polysaccharides; membranous growth generally results from profuse formation of filaments.

Color: any distinctive colors, such as yellow, orange and red are recorded. The presence of red, orange or yellow non-diffusible carotenoid pigments is characteristic of certain genera, for instance, *Phaffia*, *Rhodosporidium*, and *Sporidiobolus*. Other yeasts, such as *Metschnikowia pulcherrima*, certain *Kluyveromyces* species, and some adenine-requiring mutants of *Saccharomyces*, produce diffusible, non-carotenoid Bordeaux-red pigments. The majority of yeasts, however, produce growth which ranges in color from whitish through cream to buff.

Color charts are not used by the majority of yeast taxonomists and so the color terminology used in descriptions of yeasts is not consistent. The adoption of a standard terminology such as used in *A Mycological Colour Chart* (Rayner 1970) is strongly recommended.

Surface: whether glistening or dull, smooth, rough, sectored, folded, ridged, or hirsute. Strains which are smooth when first isolated sometimes become rough when kept on agar. This change is, in some cases, accompanied by a change in texture from butyrous to membranous. Changes of this kind have often been observed at the CBS Culture Collection in strains of *Candida albicans* and *C. tropicalis* which had been maintained on malt agar and glucose–peptone–yeast extract agar for several years. The smooth form could usually be recovered by incubating subcultures at a

temperature considerably higher than that normally used, i.e., 37 or 40°C.

Elevation: whether the growth is flat or raised.

Margin: whether the edge of the streak or colony is smooth, entire, undulating, lobed, erose, or fringed with filaments.

Media

(1) Malt extract. See p. 79.

(2) 2% (w/v) Glucose–peptone–yeast extract broth. Dissolve 20 g of glucose, 10 g of peptone, and 5 g of yeast extract in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min.

(3) 2% (w/v) Glucose–peptone–yeast extract agar. Prepare as in (2) and add 20 g of agar per liter of medium. Sterilize by autoclaving at 121°C for 15 min.

(4) Leeming and Notman agar. See p. 79.

(5) *Cyniclomyces* medium. Dissolve 10 g of yeast autolyzate (p. 90), 40 g of glucose, 10 g of proteose peptone, and 20 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min. Melt the agar just before use, cool to approximately 45°C, then adjust the pH with 1 N HCl to between 3.5 and 4.5 (approximately 4.5 ml is required for each 100 ml of medium), and pour into petri dishes. Yeast infusion can be substituted for the water and yeast autolyzate.

(6) Malt agar. See p. 79.

(7) Malt (extract) agar–2% calcium carbonate. Finely powdered calcium carbonate is sterilized by dry heat (160–180°C) for 2 h and 20 g is added to 1 liter of malt agar. Sterilize by autoclaving at 121°C for 15 min. When preparing slants and plates, the medium must be gently agitated until the agar is on the point of setting, otherwise most of the calcium carbonate will settle to the bottom of the tube or dish.

(8) Morphology agar. The composition of this chemically defined medium is given in Table 14 (p. 99). This medium, marketed by Difco Laboratories as Bacto Yeast Morphology Agar, should be prepared according to the instructions on the container.

3.1.2.2. Filamentation: Mature buds can either become detached as discrete cells or remain attached to the parent cell and give rise to either agglomerations or chains of cells. The tendency of some yeasts to form chains of cells results in the formation of pseudohyphae. A pseudohypha is, therefore, defined as a filament composed of a chain of cells which has been formed by budding.

Pseudohyphae may be either rudimentary, in which case they consist of cells of similar size and shape, or they may be differentiated into elongated cells, each of which may produce blastospores in a regular and more or less characteristic arrangement. The arrangement of blastospores was used for the differentiation of certain genera in earlier taxonomic systems (see van der Walt 1970a). The form of pseudohyphae can be markedly affected by cultural conditions (van der Walt 1970a).

An unusual type of pseudohypha is restricted to some species of *Dekkera* and is called blastese. This term describes the so-called germination of blastospores in which the germ-tube results in slender filamentous aseptate elongations (Langeron and Guerra 1939, 1940). This term has been broadened to include the formation of pseudohyphae consisting of a single filamentous cell which does not form septa, but which sometimes branch.

Some yeasts produce true septate branching hyphae under suitable conditions. True septate hyphae elongate by continuous growth of the hyphal tip followed by the formation of septa. Septation lags behind the growth of the hyphal tip to such a degree that the terminal cell, measured from tip to first septum, is normally longer than the preceding cell, measured from first to second septum (Wickerham 1951). The fine structure of hyphal septa varies among taxa. Light microscopy does not reveal much detail of the hyphal septa of filamentous yeasts except the presence of visible pore bodies.

Since budding and fission sometimes occur concurrently, it is frequently difficult to distinguish true hyphae, pseudohyphae, and intermediate forms. Wickerham (1951) applied three criteria to recognize types of hyphae. He based these criteria on observations of the terminal cells of the filaments. Firstly, true hyphae usually have refractive straight septa that can generally be differentiated by their greater thickness and refractivity from the edges of vacuoles. There is little or no constriction at the septum. The terminal cells are considerably longer than the cells immediately preceding them. Secondly, pseudohyphae do not have discernible septa, and the ends of intercalary cells are curved and not refractive. There are marked constrictions where the cells join. The terminal cell is, as a rule, shorter than or nearly as long as the adjacent cell. It is rare to find a pseudohypha with a terminal cell that is distinctly longer than the adjacent cell. Thirdly, only a small proportion of cells are separated by septa in intermediate forms.

Hyphae of some yeasts break up or disarticulate to form one-celled arthrospores (also called arthroconidia) (Fig. 23). Arthrospores formed in this way on solid media are frequently arranged in a characteristic zigzag fashion.

Hyphae may show modifications apart from simple branching. They include the formation of lateral conidia on differentiated conidiogenous cells. The presence of conidia on denticles is a characteristic of *Stephanoascus* species and *Pichia burtonii*. Conidiogenous cells showing repetitive monopolar budding are a characteristic of *Ambrosiozyma cicatricosa*.

Hyphae of some species form clamp connections. Clamps arise by outgrowths on the hypha at cell division and connect the two cells that result from the division. Their purpose is to ensure that daughter nuclei, that form during nuclear division, are both transferred to the new cell. One daughter nucleus migrates through the clamp and the other through the hypha. Some strains have incomplete clamps, i.e., the clamp does not connect to the new hyphal cell. This occurs when the nuclei fail to divide. Clamps are characteristic of the dikaryotic

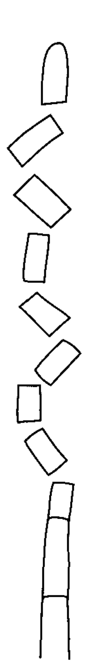


Fig. 23. Arthrospores (from van der Walt and Yarrow 1984a).

phase of basidiomycetous taxa. Hyphae are sometimes joined by a process in which there is the fusion of branches of the same or different hyphae, this is called anastomosis. Anastomosing hyphae are a characteristic of *Ambrosiozyma platypodis*.

Methods. The media most commonly employed in routine testing for the formation of filaments are: corn meal (maize) agar, morphology agar, and potato-dextrose agar. Some clinical laboratories use rice agar.

(1) Slide cultures. A petri dish containing a U-shaped glass rod supporting two glass microscope slides is sterilized by dry heat at 160–180°C for 2 h. The agar is melted and poured into a second petri dish. The glass slides are quickly removed from the glass rod with a flame-sterilized pair of tweezers and dipped into the molten agar, after which they are replaced on the glass rod. The layer of agar on the back of the slide is wiped off after the agar has solidified. In many laboratories the molten agar is poured onto the glass slides to form a thin layer.

After the surface of the agar has dried, the yeast is lightly inoculated in either one or two lines along each slide and a sterile cover slip is placed over part of each line. A little sterile water is poured into the petri dish to prevent the agar from drying out. The culture is then incubated for up to 21 days at either room temperature or another temperature suitable for the strain. The culture is examined microscopically at intervals of a few days for the formation of filaments along the edges of the streak, both under and around the cover slip.

(2) Dalmau plates. Agar is poured into petri dishes, which are then put aside for a day or two to allow the surface to dry. The yeast is inoculated as a single streak near one side of the plate (for example from the ten o'clock position to the two o'clock position), and as two points near the other side of the plate (for example at the four and eight o'clock positions). A sterile cover slip is placed over the center of the streak and another over one of the point inoculations. The cultures are incubated and examined in the same way as slide cultures.

Media

(1) Corn meal (maize) agar. Heat 42 g of maize in 1 liter of demineralized water at 60°C for 1 h, filter through paper then restore the volume to 1 liter by adding water. Add and dissolve 12 g of agar. Sterilize at 121°C for 15 min. Commercial products are available from various suppliers.

(2) Potato-dextrose agar. An infusion of potatoes is prepared by soaking 300 g of washed, peeled, and finely grated or homogenized potato in 900 ml of water overnight in a refrigerator. The resulting infusion is filtered through cheese cloth and autoclaved at 110°C for 1 h.

Dissolve 20 g of glucose and 20 g of agar in 230 ml of potato infusion and 770 ml of demineralized water. Sterilize by autoclaving at 121°C for 15 min. Commercial products are available from various suppliers.

(3) Rice agar. Simmer 20 g of *unpolished* rice in 1 liter of water for 45 min, filter, and add water to restore the volume to 1 liter. Add and dissolve 20 g of agar. Sterilize by autoclaving at 121°C for 15 min. Commercial products are available but the results obtained with them are usually inferior to those obtained with the medium freshly prepared with rice infusion.

(4) Morphology agar, see p. 82.

3.1.2.3. Formation of asexual endospores: Asexual endospores are not commonly formed, but they have been observed in strains of the genera *Trichosporon, Candida, Cryptococcus*, *Oosporidium, Cystofilobasidium*, and *Leucosporidium*. Endospores are vegetative cells which are formed within discrete cells and hyphae. Unlike chlamydospores and ascospores, endospores cannot be stained selectively. They are usually observed in old cultures on YM agar, malt agar, potato-dextrose agar, and corn meal agar kept at room temperature.

No special media have been devised to stimulate the development of endospores. The publication by do Carmo-Sousa (1969b) should be consulted for a more detailed discussion of endospores.

3.1.2.4. Formation of chlamydospores: The chlamydospore has been defined as a thick-walled non-deciduous, intercalary or terminal, asexual spore formed by the rounding off of a cell or cells (Ainsworth 1971). The asexual nature of the chlamydospore distinguishes it from the teliospore of the Sporidiales and Ustilaginales from which the basidium is produced.

As chlamydospores are generally rich in lipids, they are well adapted to maintain vitality through periods of dormancy. Mature chlamydospores have particular

affinities for certain dyes and, in contrast to normal vegetative cells, are markedly acid-fast on staining, a characteristic shared by ascospores (van der Walt 1970a). It can be observed in older cultures that these cells shed their outer layers just before or during germination.

Chlamydospores are characteristic of *Candida albicans* and *Metschnikowia* species, but are also occasionally noticed in old cultures of other taxa on agar, including some *Trichosporon* and *Cryptococcus* species. However, chlamydospores fulfill a dual function in the genus *Metschnikowia* as they germinate giving rise either to budding diploid cells or to asci.

The production of chlamydospores by *Candida albicans* is best observed in slide cultures on rice agar; corn meal agar also gives good results with some strains. Some laboratories add Tween 80 (1%) to these media.

3.1.2.5. Formation of germ tubes by *Candida albicans*: The formation of germ tubes is accepted by many medical laboratories as a reliable means of rapidly identifying *Candida albicans* (Stenderup and Thomsen 1964, Ahearn et al. 1966, Joshi and Gavin 1974). A germ tube is a thin filamentous outgrowth without a constriction at its point of origin on the cell. The formation of germ tubes is influenced by temperature, inoculum, medium, and strain. Ogletree et al. (1978) evaluated the various techniques of inducing their formation.

Method. A simple method of testing for germ tubes is to suspend cells from a 24-hour-old culture (10^5–10^6 cells per ml) in either normal blood serum or egg albumin. The cells are examined microscopically after incubation at 37°C for 1–3 h.

3.1.2.6. Formation of ballistospores: The formation of forcibly discharged asexual spores, known as ballistospores or ballistoconidia, is a specialized mode of reproduction which is encountered in some basidiomycetous genera, such as *Sporidiobolus, Bullera,* and *Sporobolomyces*. Ballistospores are produced on sterigmata that protrude from vegetative cells (Fig. 24) and are discharged into the air by the so-called droplet mechanism (Kluyver and van Niel 1924).

Methods. Ballistospores are generally detected as a mirror image of the culture formed by the discharged spores on the lid of an inverted petri dish. Suitable media are: corn meal agar, malt agar, morphology agar, and potato-dextrose agar. Do Carmo-Sousa and Phaff (1962) described the following procedure. Inoculate a petri dish containing 10 ml of corn meal agar in two lines at right angles across the diameter. The dish is inverted over the bottom of another petri dish containing malt agar on which a sterile slide has been placed. One of the lines is positioned over the slide and the halves of the two dishes are taped together. The culture is incubated at 18 to 20°C. Discharged spores germinate to form colonies on the bottom dish and are collected on the glass slide, which can be removed for examination under the microscope.

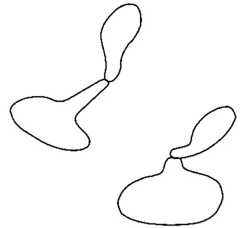


Fig. 24. Ballistospores (from van der Walt and Yarrow 1984a).

Media

(1) Malt agar, see p. 79.
(2) Potato-dextrose agar, see p. 83.
(3) Corn meal agar, see p. 83.
(4) Morphology agar, see p. 82.

3.2. Characteristics of sexual reproduction

Many yeasts reproduce sexually, resulting in an alternation of generations with the formation of characteristic cells in which reduction division takes place. In the ascogenous yeasts, the site of meiosis is the ascus where the haploid generation of ascospores is formed by so-called 'free-cell' formation, i.e., the process by which the cytoplasm surrounding the meiotic nuclei becomes enveloped by a wall. In the basidiomycetous yeasts, reduction division is restricted to either the teliospore or the basidium on which the haploid basidiospores are formed externally.

A yeast that forms either asci or basidia has been referred to as either a perfect yeast or as having a perfect state. A yeast that does not form either asci or basidia has been referred to as an imperfect yeast or as an imperfect state. In current mycological parlance the perfect state is termed the teleomorphic state or *teleomorph*, the imperfect state is termed the anamorphic state or *anamorph*, and the combined states are termed the *holomorph*. The teleomorph and anamorph of the same yeast can have different names, for instance *Pichia jadinii* (teleomorph) and *Candida utilis* (anamorph) are states of the same yeast. The holomorph has the same name as the teleomorph, i.e., *Pichia jadinii* in this example. However, only the teleomorph of many species has been named, the anamorph has not been named separately in an imperfect genus.

3.2.1. Characteristics of ascospore formation: Ascogenous yeasts can be either homothallic or heterothallic, and the vegetative phase is normally either diploid or haploid or a mixture of the two. The existence of higher degrees of ploidy has also been reported.

In haploid homothallic yeasts, plasmogamy, karyogamy, and meiosis occur within the zygote, which is generally formed by two vegetative cells fusing. This diplophase is transient, being restricted to the diploid zygote within which the ascospores are formed. Such a life cycle is termed haplontic.

One mode of diploidization involves a haploid vegetative cell which undergoes mitosis and forms a bud. The bud remains attached to the parent cell which is converted into an ascus in which usually 1 to 4 ascospores

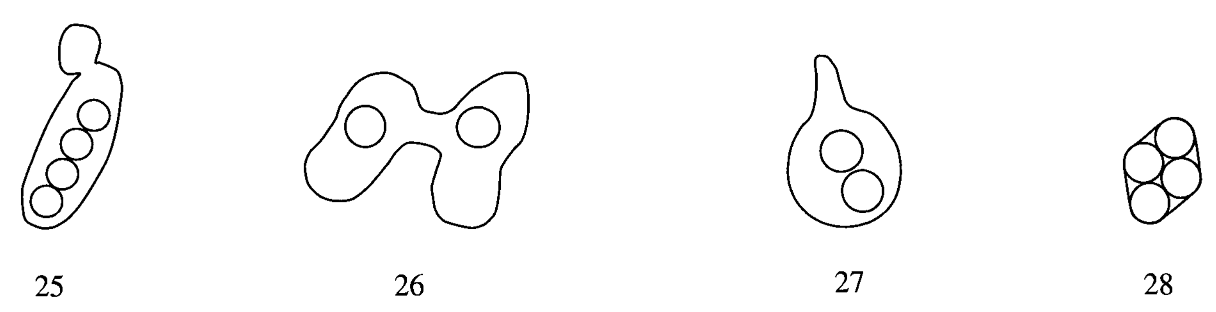


Fig. 25. Ascus formed by parent–bud (mother–daughter) cell conjugation. Fig. 26. Conjugated ascus. Fig. 27. Ascus with abortive conjugation tube. Fig. 28. Unconjugated ascus. (From van der Walt and Yarrow 1984a.)

are formed (Fig. 25). Asci bearing such vestigial buds are found in the genus *Debaryomyces*, and some species of the genera *Torulaspora* and *Pichia*. Kreger-van Rij and Veenhuis (1975a, 1976b) and Kreger-van Rij (1977a) maintain that the bud is abstricted, with subsequent dissolution of the cross wall which separates the two cells, before the two daughter nuclei fuse. Van der Walt et al. (1977) take the view that abstriction of the bud is not a prerequisite of the process. This mode of ascus formation is referred to as conjugation between a cell and its bud (mother–daughter cell conjugation) or bud-meiosis. As the process involves the fusion of two sister nuclei, it does not constitute heterogamy. Strains of species in which diploidization is effected exclusively by this process would be predominantly inbreeding because conjugation between a cell and its bud does not involve the fusion of independent cells. A process comparable to cell–bud conjugation appears to operate in the genus *Nadsonia*, where karyogamy is effected by the fusion of the nuclei of a bud and its parent. The contents of the zygote move into a third bud at the opposite pole. The second bud, which is abstricted by a septum, becomes the ascus.

Diploidization may also be brought about by the fusion of two independent haploid cells. The cells themselves may fuse giving rise to amoeboid conjugated asci as in *Schizosaccharomyces*. Alternatively, the cells may form elongated copulatory processes, or conjugation tubes, which fuse to give dumbbell-shaped asci (Fig. 26) as in *Zygosaccharomyces*. Occasionally the conjugation tubes fail to fuse. Cells bearing such abortive conjugation tubes nevertheless sometimes convert into asci with 1 or 2 spores (Fig. 27). In this event, it is presumed that the haploid nuclei of such cells undergo mitosis and the sister nuclei fuse in a manner comparable to that in conjugation between a parent cell and its bud. The process constitutes somatogamous autogamy because the protuberance is not abstricted from the cell. Asci bearing either vestigial buds or abortive conjugation tubes may be formed concomitantly, for instance in *Debaryomyces* and *Torulaspora*.

In homothallic yeast strains with a diploid vegetative phase, a single diploid vegetative cell may undergo reduction division and become an unconjugated ascus (Fig. 28) as in the genus *Saccharomyces*. The diploid condition is soon restored, either by germinating ascospores conjugating within the ascus, or by daughter nuclei fusing autogamously at the conclusion of the first mitotic division within a germinating ascospore (Winge and Laustsen 1937). The latter process has been referred to as direct diploidization or autodiploidization. The haploid phase is of very short duration in such a cycle and is restricted to the ascosporal stage. A life cycle characterized by these features is referred to as diplontic.

When some zygotes, in the case of the haplontic cycle, proceed to reproduce mitotically, or when, in the case of the diplontic cycle, diploidization of the ascospores is delayed, the result is a vegetative phase consisting of both diploid and haploid cells. Such a mixed vegetative phase then gives rise to conjugated as well as unconjugated asci.

The diploid cells of heterothallic strains are normally heterozygous for the mating-type genes and bisexual. The existence of unisexual diploid strains has been reported (Wickerham 1958, Oshima and Takano 1972). The asci remain unconjugated and unisexual haploid ascospores of both mating types are formed if the diplophase is stable. Ascospores of opposite mating type either conjugate within the ascus giving rise to the diplophase, as in *Saccharomycodes*, or the ascospores germinate giving haploid vegetative cells which can be of opposite mating types. Normally the diplophase can only be restored if conjugation of haploid cultures of opposite mating type occurs, as in *Pichia*. Active cultures of mating types are not invariably stable and may revert to sporulating cultures as a result of mutation of the mating-type alleles (Hawthorne 1963, Takano and Oshima 1967, 1970). Some species have both heterothallic and homothallic strains; *Pichia membranifaciens* and *Saccharomyces cerevisiae* are examples. Heterothallism may also be associated with sexual agglutination, as in *Pichia canadensis* (*Hansenula wingei*) and *Saccharomyces kluyveri* (Wickerham 1956, 1958), in which cells of opposite mating types agglutinate when mixed. Agglutination in *Pichia canadensis* has been shown to be mediated by complementary glycoproteins present on the surface of cells of the opposite mating types (Brock 1959, Taylor 1965, Crandall and Brock 1968).

Winge and Roberts (1954) reported the formation of binucleate ascospores in yeasts which normally remain haploid in single-spore cultures. Wickerham (1958) reported the formation of triploids and tetraploids in

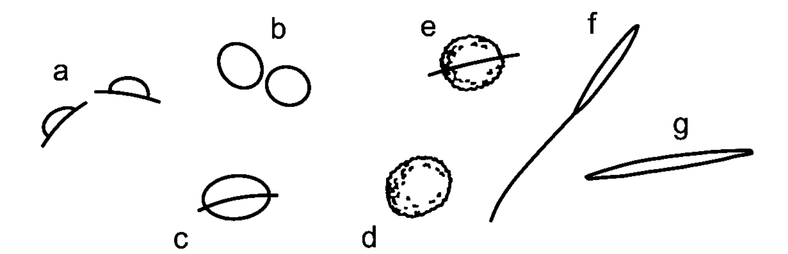


Fig. 29. Representative ascospores found among the yeasts: (a) hat-shaped, *Pichia anomala*; (b) spheroidal and smooth, *Saccharomyces cerevisiae*; (c) saturn-shaped and smooth, *Williopsis saturnus*; (d) spheroidal and roughened, *Debaryomyces hansenii*; (e) saturn-shaped and roughened, *Debaryomyces occidentalis*; (f) elongated with terminal appendage, *Eremothecium coryli*; (g) needle-shaped, *Metschnikowia reukaufii*.

Saccharomyces kluyveri by the conjugation of unisexual diploid cells with unisexual haploid and diploid cells of the opposite mating type.

The mycelial phase of filamentous yeasts may be either haploid or diploid, and this determines how the ascus is formed. The fusion of two hyphae of opposite sex, or anastomoses of lateral branches of two such hyphae, precedes ascus formation in the case of the haploid heterothallic species *Stephanoascus ciferrii*. The asci of yeasts with diploid hyphae are borne either in characteristic clusters (as in *Ambrosiozyma monospora*), or in terminal chains (as in *Ambrosiozyma platypodis*), or hyphal units can be converted into intercalary asci (as in *Saccharomycopsis capsularis*).

The form of the asci can characterize a genus; for instance, in *Lipomyces* the asci are sac-like appendages or structures, in *Metschnikowia* they are long and clavate. Asci are either persistent, as in *Saccharomyces* and *Zygosaccharomyces*, rupturing only when the spores germinate, or evanescent, as in *Kluyveromyces* and *Clavispora*, the wall lysing and freeing the ascospores. Liberated ascospores tend to aggregate in masses.

Ascospores vary in the number present in the asci, in shape, in size, in ornamentation, and in color. The number of ascospores in an ascus can be one or many, though two to four are the most common. Asci with either one or two ascospores are normal in *Lodderomyces*, and some species of *Debaryomyces*. Asci with more than eight spores are usually characteristic of *Lipomyces* and some species of *Kluyveromyces*, although they have occasionally been observed in strains of some species of *Pichia* and *Saccharomyces cerevisiae*.

The shape of ascospores varies widely and includes globose, ellipsoidal, cylindrical, reniform, crescentic, clavate, hat-shaped (galeate), cap-shaped, saturnoid, walnut-shaped, falcate, needle-shaped, and spindle-shaped with whip-like appendages (Fig. 29). The surface may be smooth or rough. However, surface ornamentation, brims and ledges may be reduced to such an extent that they cannot be detected by light microscopy. The morphology of the ascospores is given particular diagnostic value in the keys. By way of example, cap-shaped ascospores are diagnostic of the genus *Wickerhamia*, and spindle-shaped ascospores of *Eremothecium coryli*. However, it should not be forgotten that variation in the shape of ascospores has been observed within a species. An example is *Pichia ohmeri*, where both hat-shaped and globose ascospores have been found depending on the strains paired.

The ascospores may be pigmented in genera such as *Lipomyces, Nadsonia, Pichia, Saccharomycopsis,* and *Debaryomyces* and, as a result, sporulating cultures assume an amber, brown, or reddish-brown color. Ascospores are generally acid-fast, a notable exception being the spores of *Schizosaccharomyces*. However, the spores of some species of this genus stain blue with Lugol's iodine owing to the presence of amyloid substances.

Ascosporulation is generally induced under conditions which restrict vegetative growth. However, it is sometimes important that cells should be well nourished and growing vigorously on a rich medium when transferred to such conditions, though some strains sporulate without any special preparation. A variety of media have been specially formulated to induce sporulation, probably the most commonly used are: malt agar, acetate agar, Gorodkowa agar, V8 vegetable juice agar, YM agar, corn meal agar, and carrot wedges. Most of these media do not contain much carbohydrate and as a consequence they support little vegetative growth.

Some genera appear to sporulate best on a particular medium: acetate agar has been recommended for *Saccharomyces* (Adams 1949, Fowell 1952, Kleyn 1954, McClary et al. 1959), and dilute V8 agar for *Metschnikowia* (Pitt and Miller 1968); many strains of *Pichia* sporulate on malt agar. Some strains of *Zygosaccharomyces rouxii* are reported to sporulate best on media containing 2% sodium chloride (Wickerham and Burton 1960). Sporulation of many strains of the genus *Lipomyces* is favored by dilute media at low temperatures (15–20°C). Temperature can affect ascus formation markedly. Temperatures between 20 and 25°C are suitable for most yeasts. Nevertheless, strains of *Debaryomyces hansenii* generally sporulate best at 20°C or slightly lower, and many strains of the genus *Metschnikowia* require temperatures between 12 and 17°C.

Some yeasts sporulate rapidly, i.e., within 48 hours, especially when first isolated; others may require much longer, up to 6 weeks or more. The ability to sporulate sometimes declines when a strain is maintained in the laboratory, and may even be lost altogether. This occurs rapidly in some isolates, perhaps after 2 or 3 subcultures, whereas other strains may be kept for many years without any apparent decline in their ability to form spores.

Methods. The strain to be examined is brought to active growth by cultivating it on a rich medium at a suitable temperature for 24–48 h. Special pre-sporulation

media have been formulated for this, such as Lindegren's medium (Lindegren 1949) and grape juice, although a general cultivation medium such as glucose–peptone–yeast extract agar serves just as well in most cases. Tubes of sporulation media are lightly inoculated from this culture and incubated at suitable temperatures as mentioned above. Preparations of material from the cultures are examined under the microscope after 2 or 3 days, 1 week, and then at weekly intervals for at least 6 weeks. Either water mounts or stained heat-fixed preparations can be used. If asci are not found after this time, the strain is either unable to sporulate or is a mating type that needs to be mated with a strain of the opposite sex.

Staining procedures. A heat-fixed preparation is flooded with a solution of 0.5% malachite green and 0.05% basic fuchsin and heated to steaming for 1 min, washed thoroughly in flowing water and blotted dry. Wickerham (1951) recommends flooding a heat-fixed preparation with a 5% solution of malachite green, heating to 80°C for 3–5 min, washing for 30 seconds, and counter staining with a 0.5% solution of safranine for 10 seconds.

Media

(1) Lindegren's pre-sporulation medium. This medium contains 10 ml of beet-leaf extract (100 g per 100 ml of boiling water), 10 ml of beet-root extract (100 g per 100 ml of boiling water), 35 ml of canned apricot juice, 16.5 ml of grape juice, 2 g of dried baker's yeast, 2.5 ml of glycerol, 1 g of calcium carbonate, and 3 g of agar. Water is added to give a final volume of 100 ml. The medium is sterilized by autoclaving at 121°C for 15 min.

(2) Grape juice pre-sporulation medium. Freshly expressed juice of any variety of grape is diluted to a density of 8–10° Balling (using a flotation meter). It is sterilized in flowing steam or by autoclaving at 110°C for 10 min.

(3) Acetate agar 1 (Fowell 1952). Dissolve 5 g of sodium acetate trihydrate in 1 liter of water and adjust the pH to between 6.5 and 7.0 before adding and dissolving 20 g of agar. Sterilize by autoclaving at 121°C for 15 min.

(4) Acetate agar 2 (McClary et al. 1959). Dissolve 1 g of glucose, 1.8 g of potassium chloride, 8.2 g of sodium acetate trihydrate, 2.5 g of yeast extract, and 15 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min.

(5) Corn meal agar, see p. 83.

(6) Gorodkowa agar. Dissolve 1 g of glucose, 5 g of sodium chloride, 10 g of peptone, and 20 g of agar in 1 liter of tap water. Sterilize by autoclaving at 121°C for 15 min. A variant of this medium which is used in some laboratories contains 2.5 g glucose, and 10 g of meat extract is substituted for the peptone.

(7) Gypsum blocks and wedges. Mix 8 parts of gypsum (calcium sulfate hemihydrate) with 3 parts of water. Cast the paste into cylindrical or wedged-shaped cylindrical forms 3–4 cm high. After they have set, the blocks are placed in suitable sterile glass dishes with lids and heated to 110–120°C for at least 2 h. Before use, either sterile water or a solution of mannitol and phosphate is added to a depth of about 1 cm. The mannitol and phosphate solution is prepared by adding 2 ml of a 5% solution of K_2HPO_4 to 18 ml of a 1% solution of mannitol. The gypsum may also be prepared in tubes. Gypsum and water are mixed to a creamy paste which is poured into test tubes through a funnel. The tubes are plugged with cotton-wool, slanted and the gypsum allowed to harden for 24–48 h at 50°C. The slants are sterilized by autoclaving at 121°C for 15 min.

(8) 5% Malt extract agar (Wickerham 1951). Dissolve 20 g of agar in 1 liter of distilled water, then add 50 g of powdered malt extract (Difco). Sterilize by autoclaving at 115°C for 15 min. As little heat as possible should be used when melting the sterile medium.

(9) Oatmeal agar. Boil 40 g of oatmeal in 1 liter of water for 1 h and then filter through cheesecloth. Add enough water to restore the volume to 1 liter and then add 15 g of agar. Sterilize by autoclaving at 121°C for 15 min.

(10) Potato-dextrose agar, see p. 83.

(11) Restricted growth (RG) medium (Herman 1971a). Dissolve 0.2 g of yeast extract, 0.2 g of peptone, 1.0 g of glucose, and 20 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min.

(12) Rice agar, see p. 83

(13) Vegetable juice agar 1: V8 agar (Wickerham et al. 1946b). Suspend 5 g of compressed baker's yeast in 10 ml of water and add to 350 ml of canned V8 juice, adjust the pH to 6.8 and heat in a boiling water bath for 10 min. The pH is again adjusted until a cooled sample has a pH of 6.8. This hot medium is then mixed with a hot solution of 14 g of agar in 340 ml of water. Sterilize by autoclaving at 121°C for 15 min. The canned vegetable juice, which contains a blend of tomatoes, carrots, celery, beet, parsley, lettuce, spinach, and watercress, can be obtained at many food shops and is marketed under the name "V8 Vegetable Juice" by Campbell Soup Company, Camden, NJ, USA.

(14) Vegetable juice agar 2 (Mrak et al. 1942a). Either mince or finely grate equal weights of washed unpeeled carrots, beet roots, cucumbers, and potatoes and mix with an equal weight of water. Autoclave the mixture at 115°C for 10 min and express it through cheesecloth. The extract has a pH of approximately 5.7. Add 2% (w/v) dried baker's yeast and 2% (w/v) agar to the extract. Sterilize by autoclaving at 121°C for 15 min.

(15) Dilute V8 agar (Pitt and Miller 1968). Mix a can of V8 juice with an equal volume of demineralized

water and adjust the pH to 5.5 with sodium hydroxide before filtering through Whatman No. 1 paper. The filtrate is then diluted 1:2, 1:9, 1:19 as required and solidified with 2% (w/v) agar. Sterilize by autoclaving at 121°C for 15 min.

(16) Vegetable wedges. Wedges of either carrot, potato, beet, cucumber or turnip can be used. The vegetables are thoroughly cleaned by washing and then long cylinders about 1 cm in diameter are cut out of them with either a cork borer or apple corer. The cylinders are cut obliquely to make wedges, rinsed in cold water and put into a glass tube with a little water to prevent drying. Sterilize by autoclaving at 115°C for 10 min.

(17) Water (aqueous) agar. Dissolve 20 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min.

(18) YM agar, see p. 79

(19) YM–2% sodium chloride agar. This medium is prepared by adding 20 g of sodium chloride to 1 liter of YM agar.

(20) Yeast extract–2% glucose agar. Dissolve 5 g of yeast extract, 20 g of glucose, and 20 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min.

(21) Yeast infusion agar. Dissolve 15 g of agar in 1 liter of yeast infusion. Sterilize by autoclaving at 121°C for 15 min.

3.2.2. Characteristics of basidiospore formation: The basidiomycetous yeasts occur as either a budding haplophase, a dikaryotic hyphal phase, or a self-sporulating diplophase. Septate dikaryotic hyphae with clamp connections are characteristic of the sexual states of basidiomycetous yeasts, but they are not always formed (Fig. 30).

Sexual reproduction in the basidiomycetous yeasts is either heterothallic or homothallic. The incompatibility system in the heterothallic species can be either bipolar or tetrapolar and the dikaryotic hyphae are produced by one of the conjugants after a pair of compatible cells have mated. The dikaryotic hyphae eventually form large inflated, frequently lipid-rich, clamped cells in which karyogamy occurs. These cells have been interpreted as probasidia because of this function. They are intercalary, lateral, or terminal, and are sometimes thick walled. Two kinds of homothallism are found in the homothallic or self-fertile strains, which are termed primary and secondary homothallism (Fell 1974). The hyphae are uninucleate and lack clamp connections in strains with primary homothallism, whereas they are dikaryotic and have clamps in those with secondary homothallism. The manner in which the large lipid-rich cells subsequently develop into basidial structures differs widely between taxa.

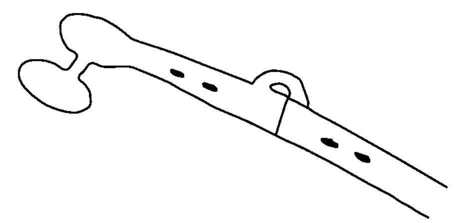


Fig. 30. Conjugated cells producing dikaryotic hypha with a clamp connection.

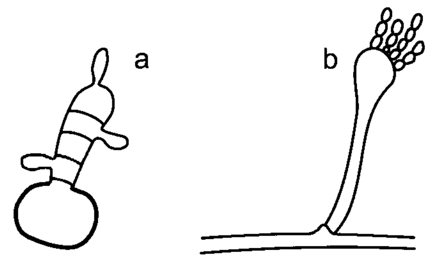


Fig. 31. Teleomorphs of basidiomycetous yeasts: (a) germinating teliospore with septate metabasidium bearing basidiospores, *Rhodosporidium toruloides*; (b) non-teliospore species with bulbous basidium bearing basidiospores, *Filobasidiella neoformans*.

The terminology of the basidium has not been standardized (see Donk 1973b). The thick-walled cells found in the ustilaginaceous genera *Rhodosporidium* and *Leucosporidium* have been referred to as teliospores, teleutospores, and ustospores (Fig. 31a). These spores are of various shapes, ranging from globose, through ovoidal to angular, and are sometimes pigmented as in *Rhodosporidium toruloides*. The teliospore forms a germ tube, called a metabasidium or promycelium, after maturing and passing through a period of dormancy. The diploid nucleus in *Leucosporidium scottii* migrates into the promycelium where it undergoes reduction division and the four haploid nuclei are distributed throughout the promycelium. However, in *Rhodosporidium toruloides,* meiosis occurs in the teliospore and the four haploid nuclei migrate into the promycelium. The promycelium then forms transverse septa which separate the haploid nuclei. Each nucleus then divides mitotically and one of these nuclei migrates into a bud that usually develops laterally on each of the promycelial cells. These haploid sessile buds are termed sporidia or basidiospores. The genetic factors controlling compatibility segregate during meiosis, with the result that the sporidia give rise to yeast phases of different mating types in heterothallic strains. In some strains of *Mrakia frigida* and *Cystofilobasidium* spp., the promycelium does not become septate and the sporidia develop terminally (Fell and Phaff 1970).

Both terminal and lateral basidia are formed on hyphae with clamp connections in *Filobasidium*. Thin-walled cells, slightly broader than the hyphae bearing them, elongate to form long slender non-septate metabasidia which taper apically. The tip is inflated and bears 6–8 sessile basidiospores arranged in a characteristic petal-like whorl (Fig. 31b). The basidiospores give rise to yeast phases of opposite mating types. In the genus

Filobasidiella, the basidia generally arise in groups either terminally or laterally on septate dikaryotic hyphae. The basidia are slender non-septate elongations with swollen apices with up to 20 haploid basidiospores in acropetal chains that arise from each of 4 sites on an apex. Karyogamy and meiosis occur in the swollen apices (Kwon-Chung 1980). The sessile basidiospores give rise to yeast phases of opposite mating types.

Methods. The heterothallic basidiomycetous yeasts are more often isolated as budding haploid yeast phases than as the sexual phase. Therefore, compatible strains need to be mated to obtain the dikaryotic clamp-bearing hyphal phase. A loopful of cells of each test strain is thoroughly mixed in as small an area as possible on an agar plate or slide. The cultures are incubated and examined at frequent intervals under low magnification (60–100×) for clamped hyphae growing into the agar around colonies where mating has occurred. Cultural conditions such as temperature may have an appreciable effect on the results of the mating tests. Media such as 5% malt extract agar, corn meal agar, hay infusion agar, and sucrose–yeast extract agar are used (see Banno 1967, Fell et al. 1969, 1973, Fell and Statzell-Tallman 1980b, Kwon-Chung 1976a, 1977b).

The basidia of *Filobasidium* and *Filobasidiella*, as well as the teliospores of some ustilaginaceous species, will mature and develop further on the media on which the dikaryotic mycelium is produced. The teliospores of other ustilaginaceous yeasts fail to germinate under these conditions and special treatments have been developed for them. Usually bits of agar containing spores are soaked in demineralized water at low temperatures, sometimes for as long as 3 months, before being put on water agar. The descriptions of the individual species should be consulted for further details.

Media

(1) Corn meal agar, see p. 83.

(2) Hay infusion agar. Autoclave 50 g of decomposing hay in 1 liter of demineralized water for 30 min at 121°C and then filter. Dissolve 2 g of potassium monohydrogen phosphate and 15 g of agar in the filtrate and adjust the pH to 6.2. Sterilize by autoclaving at 121°C for 15 min.

(3) 5% Malt extract agar, see p. 87

(4) Sucrose–yeast extract agar. Dissolve 1 g of potassium dihydrogen phosphate, 0.5 g of magnesium sulfate heptahydrate, 0.1 g of calcium chloride, 0.1 g of sodium chloride, 0.5 g of yeast extract, 20 g of sucrose, 5 μg of biotin, and 40 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min.

3.2.3. Methods of isolating mating types: When dealing with strains whose perfect state or teleomorph is not known and when testing for interfertility, it is necessary to isolate mating types from haploid and diploid heterothallic species. A strain that does not produce a sexual state might be a mating type and should be tested to determine whether or not it has the ability to mate by mixing it with other non-sporulating strains of the same species. The more strains that are tested in this way the greater the chance of detecting compatible mating types. Approximately equal amounts of young actively growing cells of the test strains are mixed on the surface of sporulation media, either as agar slants or in petri dishes. The cultures are examined under the microscope for zygotes and sexual structures at intervals of about 3 days for up to 12 weeks. If sexual activity is detected, the strains are then mixed in pairs to determine which are compatible. More details can be found in the publications of Wickerham (1952, 1960, 1965a,b, 1969a), Wickerham and Burton (1952, 1954a,b, 1962), Phaff and Yoneyama (1961), and Slooff (1964).

Mating types of diploid heterothallic species are obtained by isolating single ascospores and basidiospores. This is most rapidly and reliably achieved by employing a micromanipulator when the strain produces enough sexual spores to be easily found and picked out. When the strain produces only very few ascospores, one must resort to the method of heat treatment as described by Wickerham and Burton (1954a), in which a suspension of cells from a sporulating culture are heated and samples are taken at frequent intervals and spread on agar plates. A temperature should be chosen at which vegetative cells are killed within about 5 min, after which only ascospores remain viable and the colonies arising on plates near the end of a series are derived from ascospores only. The most suitable temperature varies with the species, but generally lies in the range of 52 to 65°C.

3.3. Physiological and biochemical characteristics

Physiological properties primarily serve to describe and identify yeast species and, to a very minor extent, genera. The tests most used for routine identification purposes are fermentation of and growth on carbon sources, growth on nitrogen sources, requirements for vitamins, growth at various temperatures and on media with a high content of sugar or sodium chloride, hydrolysis of urea, and resistance to antibiotics. There is not a single standardized method for many of these tests. The results of such tests are frequently dependent on the techniques employed, therefore whichever procedure is chosen, it should be scrupulously adhered to and young vigorously growing cultures should be used as inocula.

3.3.1. Utilization of carbon compounds: There appear to be no exceptions to the rule that when a yeast strain ferments a carbohydrate it is also able to grow on it. However, the reverse does not hold true: many yeasts grow aerobically on sugars they cannot ferment. It is essential that only pure, high-grade carbohydrates are used in the preparation of the media used in these tests.

3.3.1.1. Fermentation of carbohydrates: Yeasts vary in their ability to ferment sugars as measured by

the production of carbon dioxide. Yeasts of the genera *Kluyveromyces, Saccharomyces*, and *Zygosaccharomyces*, for example, ferment, at least, glucose vigorously, whereas others, such as *Rhodosporidium* and *Sterigmatomyces*, do not noticeably ferment any sugars. Species ranging from non-fermentative to strongly fermentative are found in other genera.

Several tests have been devised to detect the production of carbon dioxide from carbohydrates (van der Walt 1970a). Durham tubes containing 2% solutions of sugar have been found to be the most useful for routine purposes. The basal medium in which the sugars are dissolved must contain adequate nutrients, so that conditions are suitable for the synthesis of the enzyme systems involved in the transport and hydrolysis of the carbohydrates. The basal medium must be free of contamination with fermentable sugars, such as trehalose in yeast infusion, because such contamination may lead to falsely positive results. The fermentation of D-glucose, D-galactose, sucrose, maltose, lactose, raffinose, and α-α-trehalose are generally tested for routine identification; other compounds such as inulin, starch, melibiose, cellobiose, and D-xylose are sometimes used.

Methods. The ability to ferment sugars is tested in Durham tubes containing 2% (w/v) solutions of sugar (except in the case of raffinose where 4% is usually used because some strains use only part of the molecule). Durham tubes are test tubes with a small inverted tube inserted to collect any gas that may be produced.

The tubes of test media are inoculated with cells from a culture which is 24–48 hours old, either in suspension or on the tip of an inoculating needle. The tests are incubated at 25°C for up to 28 days. The tubes are shaken and inspected at frequent intervals for accumulation of gas in the insert and, if used, a change of color in the indicator. The results are scored as follows, depending on the time taken to fill the insert with gas and the amount accumulating:

+, strongly positive, insert filled within 7 days;
l, delayed positive (latent), insert rapidly filled, but only after more than 7 days;
s, slowly positive, insert slowly filled after more than 7 days;
w, weakly positive, the insert is not fully filled with gas (e.g., less than $\frac{1}{3}$ full is often considered weak, whereas greater than $\frac{1}{3}$ full is positive);
–, negative, no accumulation of gas in the insert;
v, variable, some strains are positive, others are negative

Media

(1) Yeast autolyzate. Mix equal parts of compressed baker's yeast and water in a flask. A thin layer of toluene may be added. Incubate at 50–55°C for three days, stirring occasionally. Boil for a few minutes and allow to cool. Adjust the pH to 6.0 by adding 1 N KOH. The supernatant is clarified either by adding egg white or by filtering using suction. The clear filtrate represents full strength yeast autolyzate. Commercial products are available and should be prepared according to the manufacturers instructions.
(2) Yeast infusion, see p. 80.
(3) Fermentation basal medium. Wickerham (1951) formulated the following medium. Dissolve 4.5 g of powdered yeast extract and 7.5 g of peptone in 1 liter of demineralized water. Add bromothymol blue to give a sufficiently dark green color. Bromothymol blue stock solution is 50 mg/75 ml distilled water. Add 4 ml stock solution per 100 ml fermentation basal medium. Put 2-ml amounts of basal medium into tubes 150×12 mm in size, which contain small (approximately 50×6 mm) inverted tubes. Sterilize at 121°C for 15 min. Add 1 ml of concentrated, filter-sterilized sugar solution aseptically to give a final sugar concentration of 2% (w/v).

Alternatively, dissolve 2 g of sugar (4 g in the case of raffinose) in either yeast infusion or a 1.0% (w/v) solution of powdered yeast extract or autolyzate. Dispense 5–6 ml amounts of medium into 150×12 mm plugged test tubes containing a small inverted tube (Durham insert) approximately 50×6 mm in size. Sterilize by autoclaving at 121°C for 15 min. When cool after autoclaving, the insert should be full of medium, because air in the inverted tubes is displaced during autoclaving. Some laboratories use screw-capped bottles instead of plugged tubes, because they have the advantage that they can be stored longer without drying but the disadvantage of being less easy to inoculate.

3.3.1.2. Assimilation of carbon compounds: The so-called assimilation tests are in fact tests of the ability of a yeast to grow aerobically on a particular carbon compound supplied as the sole source of energy. The tests can be done either on solid media or in liquid media. However, the use of liquid media is advocated when preparing descriptions of strains and species.

The method employing tubes of liquid media was described by Wickerham and Burton (1948) and Wickerham (1951). The results are improved by agitating the tubes during incubation (Barnett and Ingram 1955, Ahearn et al. 1960, Barnett et al. 1990). Some workers incubate the tests for a period of three weeks, others for four weeks. These long incubations allow the yeast to adapt to utilize some compounds. The tests on solid media can be done in two ways. The first is the auxanographic method of Beijerinck (1889a) in which the yeast is suspended in agar in pour plates and the test sugars are spotted at intervals around the circumference. The second way is to incorporate the test compound into a nutrient agar base in petri dishes and inoculate the test yeast as either a streak or a dot on the surface. This technique was used by Shifrine et al. (1954) and Beech et al. (1955) for screening many isolates on a single plate and can be used with replica-plating. The disadvantage of these agar plates is that they tend to dry within a few days and therefore do not

detect delayed growth on carbon sources. However, they have the advantage that the results can be read after 2–4 days and that any contaminating organisms can easily be seen, which makes them very suitable for the preliminary screening of many isolates.

In the present volume, 36 carbon compounds are used in the description of each species, namely:

- Hexoses: D-glucose, D-galactose, L-sorbose;
- Disaccharides: cellobiose, lactose, maltose, melibiose, sucrose, α-α-trehalose;
- Trisaccharides: melezitose, raffinose;
- Polysaccharides: inulin, soluble starch;
- Pentoses: D-arabinose, L-arabinose, D-ribose, L-rhamnose, D-xylose;
- Alcohols: *meso*-erythritol, galactitol, D-glucitol, glycerol, *myo*-inositol, D-mannitol, ribitol, ethanol, methanol;
- Organic acids: citric acid, DL-lactic acid, succinic acid, D-gluconic acid;
- Glycosides: α-methyl-D-glucoside, salicin;
- Other compounds: D-glucosamine hydrochloride, *N*-acetyl-D-glucosamine, hexadecane.

Additional compounds are sometimes used when they are needed to discriminate between species of a particular genus; these include: 2-keto-D-gluconate, 5-keto-D-gluconate, saccharate, arbutin, D-glucuronate, xylitol, and L-arabinitol.

3.3.1.2.1. Assimilation tests in liquid medium. (See Wickerham and Burton 1948, Wickerham 1951, Barnett and Ingram 1955, Ahearn et al. 1960).

Method. It is reiterated that only pure high-grade chemicals should be used to prepare the media, as appreciable amounts of impurities may be present in some products, for instance D-glucose in maltose, and D-galactose in L-arabinose.

The tests are done in rimless test tubes (180 mm by 16 mm), either with plugs or caps. Each tube contains a standard 5 ml amount of liquid nitrogen base medium with one test substrate, except those for the negative controls, which have no carbon source added, and those for the positive controls, which contain glucose.

Suspensions of cells from young actively growing cultures are used to inoculate the tubes of test media. For this purpose the yeast is cultivated on any convenient medium such as glucose–peptone–yeast extract agar for 24–48 h, or longer if it is particularly slow growing, at a temperature at which the strain grows well. Material is taken from this culture aseptically with a sterile platinum needle or loop and dispersed in about 3 ml of liquid, taking care to avoid carrying over any of the nutrient medium. Some laboratories make suspensions in sterile distilled (or demineralized) water, others use sterile yeast nitrogen base. A white card with black lines approximately 0.75 mm wide drawn on it is held behind the tube. The suspension is diluted aseptically until the lines become visible through the tube as dark bands; this is the same as a 2+ reading as in evaluating growth tests. Each of the tubes containing the test media is inoculated with 0.1 ml of the suspension. Some laboratories dissolve the test sugars in demineralized water instead of basal medium and dispense 4.5 ml of this solution into test tubes. In this case, 2.5 ml of the suspension of yeast in nitrogen base is pipetted aseptically into 25 ml of basal medium and each tube is then inoculated with 0.5 ml of the resulting suspension. Soluble starch and inulin are sometimes prepared at single strength and sterilized by filtration.

Wickerham (1951) described a special medium to starve the inoculum. This medium is the same nitrogen base used in the tests but contains only 0.1% (w/v) of glucose. Cells from a culture grown for 24–48 hours on agar are inoculated into 10 ml of this medium in a test tube and then incubated for a further 48 hours. The culture is then diluted with basal medium until the density when measured in a photometer is equal to that imparted by either 10×10^6 cells per ml of *Saccharomyces cerevisiae* NRRL Y-567 (= ATCC 9763 = CBS 2978) or 15×10^6 cells per ml of *Candida utilis* NRRL Y-900 (= ATCC 9950 = CBS 5609 = IFO 0988). Cells at these concentrations in test tubes with a diameter of 16 mm transmit about 55% of light passing through a blue filter (420 nm) in a Lumitron photometer. The cell suspension may be diluted using a card with black lines as described above if a photometer is not available. Each tube of test medium is inoculated with 0.1 ml of the final suspension.

The tubes of inoculated test media are incubated at either 25°C or 28°C, usually for 3 weeks, though some workers extend this to 4 weeks. Strains of some species fail to grow or grow only poorly at these temperatures (see descriptions of individual species) and a suitable temperature must be used; 15°C is suitable for most psychrophilic yeasts. It is the practice in some laboratories for the tubes to be stationary, perhaps tilted to improve aeration by exposing a larger surface of liquid. However, better aeration and mixing are obtained if the tubes are agitated, giving more reliable and quicker results. This is achieved either by putting the tubes on a rotary shaker or a Rollordrum (New Brunswick Scientific Co.) or by rocking them. The angle of rocking should be as wide as possible without causing wetting of plugs or caps. T-tubes (Monod tubes) can be agitated far more energetically than test tubes (Monod et al. 1951, Barnett et al. 1990). They can be rocked to an angle of up to 90° each side of the horizontal or shaken on a reciprocating shaker. The great disadvantages of T-tubes are that they are expensive to buy and troublesome to clean, handle and store.

Results are read after 1 week and after 3 weeks; in some laboratories also after 2 weeks and 4 weeks. The degree of growth is assessed by eye by placing the tubes, after they have been shaken well to thoroughly disperse the yeast, against a white card on which lines 0.75 mm thick have been drawn approximately 5 mm apart with India ink. The result is scored as 3+ if the lines are completely obscured; as 2+ if the lines appear as diffuse bands; as 1+ if the lines are distinguishable as such but have blurred edges;

as negative if the lines are distinct and sharp edged. In those cases where the outcome is doubtful, growth may be checked by inoculating 0.1 mm of the culture into a tube of fresh test medium. After reading, several tubes of culture are tested for a starch reaction as described in a later section (p. 95).

The results are presented in the descriptions as follows:

+, positive, either a 2+ or a 3+ reading after 1 week, or 2 weeks in some laboratories;
l, delayed positive (latent), either a 2+ or 3+ reading develops rapidly, but after 2 weeks or longer;
s, slow positive, a 2+ or 3+ reading develops slowly over a period exceeding 2 weeks;
w, weakly positive, a 1+ reading;
–, negative;
(+), seldom positive (if a particular result is seldom observed, it is sometimes given in parentheses);

Various combinations may be also used, such as:

v, variable: some strains are positive, others negative;
+/w, positive or weak: all strains grow, but some of them grow weakly;
w/–, weak or negative.

Media

(1) Inoculation medium of Wickerham (Wickerham 1951). Dissolve 6.7 g of Bacto Yeast Nitrogen Base and 1 g of glucose in 100 ml of demineralized water. This 10-fold concentrated solution is filter sterilized and stored in a refrigerator. The final medium is prepared by aseptically pipetting 1-ml amounts of this basal medium into 9 ml of sterile demineralized water.
(2) Nitrogen base medium for carbon assimilation tests in liquid medium. The composition of Bacto Yeast Nitrogen Base is given in Table 14 (p. 99). A 10-fold concentrated solution of nitrogen base medium is prepared by dissolving 6.7 g of Bacto Yeast Nitrogen Base and the amount of the carbon compound equivalent to 5 g of glucose (with warming if necessary) in 100 ml of demineralized water. Filter sterilize and store refrigerated. Tubes of test media are prepared by aseptically pipetting 0.5 ml of the concentrate into 4.5 ml of sterile water in a 16×180 mm test tube.

3.3.1.2.2. Assimilation tests on agar medium. The ability to grow on various carbon sources in an agar medium may be tested either in tubes, or on agar plates by replica plating, or in auxanogram plates.

Methods

(1) Tube method. Each tube of a set containing the various carbon sources in a basal agar medium is inoculated with one drop of a suspension of cells from a young actively growing culture. The tubes are incubated at a suitable temperature and the results assessed by inspecting the cultures after 1, 2, and 3 weeks. The amount of growth on each of the test media is compared with that on a negative control consisting of the basal medium without any carbon source.

The tubes of test media are prepared by aseptically adding 0.5 ml of a 10-fold concentrated filter-sterilized solution of the test compound to 4.5 ml of sterile molten basal medium in 16×180 mm test tubes. The tubes are slanted after their contents have been carefully mixed and the agar is allowed to set.
(2) Replica plating. A set of plates, each containing one carbon source in basal agar medium, is inoculated by transferring cells from many young colonies simultaneously by "printing". The results are read by inspecting the plates and comparing the colonies with those of a negative control provided by a plate containing the basal medium without a carbon source.

The various strains to be tested are spot inoculated with a needle onto a plate of a suitable agar medium such as glucose–peptone–yeast extract agar. The plates are incubated for 24–48 hours. The size of the petri dishes determines how many strains can be put on this master plate. The dishes are marked to orientate them and the young discrete colonies are printed to each of the plates in the test set. This is achieved by picking up the colonies on a disk of sterile filter paper, or velveteen cloth, attached to the end of a wooden or metal cylinder by pressing it first on to the master plate and then on to each of the test plates in turn. Ridgeway Watt (1979) described an automatic device for replica plating.
(3) Auxanograms. Petri dishes of basal agar medium in which yeast cells are suspended are seeded with carbon sources at various points around the periphery. The results are read by inspecting the plates for an opaque zone of growth around the point where a carbon source was applied.

The basal agar medium is melted and cooled to between 40 and 45°C. A suspension of young cells in water is either added to a tube of medium, mixed and then poured into a petri dish or, alternatively, pipetted into a petri dish into which the agar medium is then poured, the plate is gently swirled to mix the contents. The petri dishes are left on a level surface for the agar to set and then allowed to stand for a few hours to allow the surface of the agar to dry. Some laboratories consider this drying step unnecessary. Three to six carbon sources can be tested on each petri dish depending on its size. The bottom of the petri dish is marked around the periphery to locate and identify the carbon sources and a small amount of each compound is deposited as aseptically as possible on the surface of the agar. The plates are examined every two days for up to a week.

Media.

(1) Basal agar medium 1 (Lodder and Kreger-van Rij 1952). Dissolve 5 g of ammonium sulfate, 1 g of potassium dihydrogen phosphate, 0.05 g of magnesium sulfate tetrahydrate, and 20 g of high-grade agar in 1 liter of demineralized water. Add either 2.0 g of

yeast extract or 10 ml of 100-fold concentrated stock vitamin solution. Dispense the medium into tubes or bottles and sterilize at 121°C for 15 min.

(2) Basal agar medium 2. Dissolve 6.7 g of Bacto Yeast Nitrogen Base and 20 g of high-grade agar in 1 liter of demineralized water. Dispense into tubes or bottles and sterilize at 121°C for 15 min.

(3) Vitamin stock solution (van der Walt and van Kerken 1961a). Dissolve 0.2 mg of biotin, 40 mg of calcium pantothenate, 0.2 mg of folic acid, 200 mg of inositol, 40 mg of niacin, 20 mg of *p*-aminobenzoic acid, 40 mg of pyridoxine hydrochloride, 20 mg of riboflavin, and 100 mg of thiamine in 1 liter of demineralized water and sterilize by filtration. The sterile solution is stored frozen at −10°C or lower.

3.3.1.2.3. Splitting of arbutin. The test for splitting of arbutin is an alternative to the growth test for determining whether or not a yeast can utilize arbutin. The aglucone moiety, hydroxyquinone, gives a brown color with any soluble ferric salts in the medium when a yeast strain hydrolyses arbutin.

Method. A slant of arbutin agar is inoculated with cells from a young actively growing culture and incubated at 25°C, or other suitable temperature. If arbutin is split, the agar develops a dark brown color, usually within 2 to 7 days.

Medium. Arbutin agar. Dissolve 0.5 g of arbutin, 1 g of powdered yeast extract, and 2 g of agar in 100 ml of demineralized water. Sterilize by autoclaving at 121°C for 15 min. After sterilization, 2 ml of a sterile 1% solution of ferric ammonium citrate is added aseptically to and mixed with the molten agar before it is dispensed into tubes or petri dishes. The tubes are slanted and the agar is allowed to set.

3.3.1.3. Assimilation of nitrogen compounds: Yeasts are capable of utilizing a wide variety of nitrogen sources. However, the utilization of nitrate, nitrite, ethylamine hydrochloride, cadaverine dihydrochloride, L-lysine, imidazole, glucosamine, creatine, and creatinine as sole source of nitrogen are the most commonly used tests. The ability to utilize nitrate was an important criterion in defining some genera, for instance the genus *Pichia* was distinguished from the former genus *Hansenula* by its inability to grow with nitrate as the sole source of nitrogen. At present, the presence or absence of this ability is mainly used as a taxonomic criterion at the species level. The methods for testing growth are similar to those described for growth on sources of carbon, using liquid or solid media, with carbon base instead of nitrogen base.

Yeasts, which grow with nitrate as the sole source of nitrogen, are also able to grow on nitrite but the reverse does not always apply. For instance, some strains of *Debaryomyces hansenii* utilize nitrite but not nitrate. Nitrite can be toxic to the yeast because nitrous acid is formed at pH values below 6, therefore media should be adjusted initially to pH 6.5, and nitrite used in low concentrations. Because of this toxicity, auxanograms are particularly suitable for testing the utilization of nitrite and of ethylamine, which also can be inhibitory at high concentrations. The use of the auxanographic method is also preferred by van der Walt (1970a) for testing the utilization of creatine and creatinine.

3.3.1.3.1. Assimilation in liquid medium. (See Wickerham 1946, 1951.)

Method. The medium used for testing the ability to utilize the various nitrogen sources is similar in composition to that used in the tests for growth on carbon compounds (Table 14, p. 99).

Tubes of test media are inoculated either with 0.1 ml of a suspension of cells in water or saline as for the carbon tests, or with a light inoculum on the tip of a needle, and incubated in the same way. A certain amount of growth is apparent after the tubes have been incubated for 3–7 days, even though the nitrogen sources may not have been utilized. This is due to reserves of nitrogen carried by the cells of the inoculum. Therefore, a second tube is inoculated with a loopful (4 mm diameter) of cells from the first. If this second tube gives a 2+ to 3+ score (as for carbon tests, see p. 91) the strain is considered capable of utilizing the nitrogen source.

An alternative method of testing for the utilization of nitrate in liquid medium is to use a chemical test for nitrate as described by Nickerson (1944a). Four tubes of assimilation medium are incubated and tested after about 3, 5, 10 and 15 days. One of the tubes will give a positive reaction for nitrite if the yeast utilizes nitrate. A few drops of reagent 1 and reagent 2 are added to test the culture (the composition of the reagents is given in the *Media and reagents* description below). The development of a distinct pink or red color indicates the presence of nitrite produced as a result of the partial utilization of nitrate. However, the absence of color may indicate that the nitrate has been completely consumed. Therefore, to test for nitrate in the medium, a small pinch of zinc powder is added to the tube in which the previously described test has just been done. Any nitrate still present is reduced to nitrite in the presence of the zinc and the characteristic pink color will develop after a few minutes.

Media and reagents.

(1) Carbon base. The stock medium is prepared at a 10-fold concentration by dissolving 11.7 g of Bacto Yeast Carbon Base, together with the required amount of the nitrogen source, in 100 ml of demineralized water. The following amounts of nitrogen sources are used: 0.78 g of potassium nitrate, 0.26 g of sodium nitrite, 0.64 g of ethylamine hydrochloride, 0.68 g of cadaverine dihydrochloride, and 0.56 g of L-lysine (van der Walt and Yarrow 1984a). The solution is sterilized by filtration and stored in a refrigerator until required. The final medium is prepared by aseptically pipetting 0.5 ml of the concentrated medium into 4.5 ml of sterile demineralized water. Tubes for negative controls are prepared by adding filter-sterilized basal medium without nitrogen source.

(2) Reagent 1. Dissolve 8 g of sulfanilic acid in either 1 liter of 5 N acetic acid (1 part glacial acetic acid to 2.5 parts of water) or 1 liter of dilute sulfuric acid (1 part concentrated acid to 20 parts of water).
(3) Reagent 2. Dissolve 5 g α-naphthylamine in 1 liter of either 5 N acetic acid or of dilute sulfuric acid (1:20), or dissolve 6 ml of dimethyl-α-napthylamine in 1 liter of 5 N acetic acid.

3.3.1.3.2. Auxanographic method. (See Lodder and Kreger-van Rij 1952.)

Method. A procedure similar to that described for the carbon tests is used (p. 92), but the basal medium lacks a nitrogen source and contains glucose as carbon source. Three nitrogen sources are tested on each plate by spotting them around the periphery, and either ammonium sulfate or peptone is used as a positive control. Sodium nitrite and ethylamine hydrochloride should be used sparingly; overdosing of the plates is best avoided by dipping an inoculating needle into saturated solutions of the salts and then touching the surface of the seeded plate with the tip. After the nitrogen sources have been introduced, the plates are incubated at a suitable temperature and inspected after 2 and 4 days for zones of growth around the sites of the nitrogen sources.

Media

(1) Basal medium I (Lodder and Kreger-van Rij 1952). Dissolve 20 g of glucose, 1 g of potassium dihydrogen phosphate, 0.5 g of magnesium sulfate (heptahydrate), and 20 g of high quality agar in 1 liter of distilled water. Sterilize by autoclaving at 121°C for 15 min.
(2) Basal medium II. Dissolve 11.7 g of Bacto Yeast Nitrogen Base and 20 g of high quality agar in 1 liter of distilled water. Sterilize by autoclaving at 121°C for 15 min.

3.3.1.4. Growth in vitamin-free medium and vitamin requirements: The ability to grow in a glucose/mineral medium devoid of all vitamins was introduced as a diagnostic property by Wickerham (1951) and extended to include the requirements for individual vitamins by van Uden and Farinha (1958) and van Uden and do Carmo-Sousa (1959).

Method. The vitamins generally employed are: *myo*-inositol, calcium pantothenate, biotin, thiamine hydrochloride, pyridoxine hydrochloride, niacin, folic acid, and *p*-aminobenzoic acid. These tests are useful for discriminating between some species, for instance, *Pichia cactophila* can readily be distinguished from *Pichia membranifaciens* by the test for growth in media without pyridoxine.

The determination of requirements for vitamins is based on whether or not a yeast can grow in a complete mineral medium devoid of either all, one or a pair of vitamins. A tube of vitamin-free medium is inoculated by needle with cells from a young vigorously growing culture and the tube is incubated at a suitable temperature with agitation for 2 or 3 days. Generally the yeast grows considerably in this first tube owing to reserves being carried over within the cell. A second tube of vitamin-free medium, and a complete set of tubes lacking individual vitamins when individual requirements are being tested, are inoculated with 1 drop of cell suspension from the first tube. The cultures are incubated and inspected after 3 and 7 days for evidence of growth.

Media

(1) Vitamin-free medium. The composition of this medium is given in Table 14 (p. 99). A 10-fold concentrated stock medium is prepared by dissolving 16.7 g of Bacto Vitamin-Free Yeast Base in 100 ml of distilled water, which is warmed slightly to effect complete solution of the ingredients.

3.3.1.5. Growth in media of high osmotic pressure: Yeasts from substrates with high sugar and salt contents are usually resistant to high osmotic pressures. Many yeast species grow well in glucose concentrations up to 40% by weight, whereas few species grow at sugar concentrations between 50 and 70%. The ability to grow with high concentrations of sugar is generally tested by growth on agar media containing 50% and 60% (w/w) glucose.

Method. Wickerham (1951) introduced the use of a liquid medium containing 5% glucose and 10% sodium chloride. Kurtzman (1990c) formulated a medium containing 16% NaCl and 5% glucose to distinguish *Zygosaccharomyces rouxii* from *Z. mellis*.

Culture media in cotton-plugged tubes and petri dishes tend to dry up quite rapidly in dry climates as the result of evaporation. Such water losses can affect the results of tests and can be reduced by incubating the tubes either in plastic bags inflated with air or with self-sealing plastic caps (Kimble P.M. Cap Closures, Kimble Inc., Toledo, OH, USA), which permit gaseous exchange but prevent water loss. A strip of plastic film is sometimes used to seal petri dishes, but this can have an adverse effect on growth by preventing aeration.

Media

(1) 50% Glucose agar. First dissolve 13 g of agar and then 500 g of glucose in 500 g of either yeast infusion or a 1% solution of yeast extract. Dispense into tubes and sterilize at 110°C for 10 min before slanting. Overheating leads to a noticeable browning because the sugar becomes caramelized; any media showing browning must be discarded.
(2) 60% Glucose agar. First dissolve 22.5 g of agar and then 600 g of glucose in 400 g of either yeast infusion or a 1% solution of yeast extract. Dispense into tubes and sterilize at 110°C for 10 min before slanting. Overheating leads to a noticeable browning because the sugar becomes caramelized; any media showing browning must be discarded.
(3) 10% Sodium chloride plus 5% glucose medium (Wickerham 1951). Dissolve 100 g of sodium chloride, 50 g of glucose, and 6.7 g of Bacto Yeast Nitrogen Base in 1 liter of demineralized water. Dispense 5-ml amounts into tubes and sterilize at 121°C for 15 min.

3.3.1.6. Growth at 37°C and at other temperatures: The strains of most yeast species grow best at temperatures between 20 and 28°C. However, some yeasts, and particularly those from restricted and specific habitats, require higher or lower temperatures. Yeasts from polar regions grow poorly at 20°C but grow well between 4°C and 15°C (Sinclair and Stokes 1965). In contrast, yeasts from mammals, such as *Cyniclomyces guttulatus*, require a temperature of 35–37°C to grow well, and 30–35°C is reported to be the optimum for *Malassezia furfur* by Leeming and Notman (1987).

Method. Some laboratories test for growth at various temperatures in liquid medium, whereas others test on an agar medium. When testing in liquid medium, a tube of either glucose–nitrogen base medium (as used for assimilation tests) or glucose–peptone–yeast extract broth is inoculated in the usual way and incubated at the chosen temperature. The result is recorded after 1 and 3 weeks (Wickerham 1951). When testing on agar medium, a slant of glucose–peptone–yeast extract agar is inoculated with cells of a young culture and incubated at the chosen temperature for 4 days and then inspected for growth.

Media

(1) 2% (w/v) Glucose–peptone–yeast extract broth, see p. 82.

(2) Glucose–peptone–yeast extract agar (GPY agar), see p. 79.

(3) Glucose–nitrogen base. Dissolve 5 g of glucose and 6.7 g of Bacto Yeast Nitrogen Base in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min.

3.3.1.7. Acid production from glucose: Yeasts produce traces of volatile and non-volatile acids in culture, however, it is only when large amounts of acetic acid are produced that acid formation has diagnostic value (Custers 1940, van der Walt 1970a). Custer's chalk medium, which contains glucose (5%) and calcium carbonate (0.5%), is used for the test and the result is determined by the calcium carbonate going into solution. Production of acid on this medium has limited use in taxonomy. It is considered characteristic of *Dekkera* and *Brettanomyces*. However, acid production is quite weak in some strains.

Some yeasts which produce citric acid, such as some *Candida* species, also give a weakly positive reaction in this test. Because of this, a weak reaction to the production of acid has to be regarded as an equivocal result.

Method. The strains to be tested are streaked onto slants or a plate of Custer's chalk medium and incubated at 25°C. The cultures are inspected at frequent intervals for up to 2 weeks for clearing of the medium around the streaks. This results from the chalk being dissolved by any acid that may have been formed.

Medium

(1) Custer's chalk medium. Dissolve 50 g of glucose, 5 g of finely powdered calcium carbonate, 5 g of yeast extract powder, and 20 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min. Cool tubes to around 45°C and agitate the medium gently to resuspend the chalk and then slant in a cool place so that the agar gels quickly before the chalk can settle.

3.3.1.8. Formation of extracellular amyloid compounds (starch formation): Aschner et al. (1945) and Mager and Aschner (1947) studied the production of extracellular starch-like material by encapsulated yeasts. They found that under suitable conditions several yeasts formed extracellular polysaccharides, which give a blue to greenish-blue color with iodine solution. One of the conditions required was that the pH of the medium be maintained below 5.0. However, some species, such as those of *Leucosporidium*, give a positive result to the starch test at pH values above 5.0.

Methods

(1) Testing in liquid culture at pH 5.6 (Wickerham 1951, 1952). Cultures for the carbon growth tests which contained sugars or polyhydric alcohols are tested for the presence of amyloid compounds. Some cultures are tested after incubation for 7 days and, if these give a negative result, others are tested after the next reading. Some laboratories prefer to use a medium containing 1% glucose. The test is done by adding one or two drops of dilute Lugol's iodine and mixing it with the culture by shaking the tube. A positive result is indicated by the development in the culture of a color varying from dark blue to green. The strain may be re-tested by cultivating it in yeast nitrogen base medium with 3% glucose if the result is doubtful.

The relatively large amount of glycogen synthesized by some yeasts interferes with the test, especially if iodine solutions stronger than 0.02 N are used. The brown color typical of glycogen may mask a weak starch reaction. The tubes should be allowed to stand at room temperature for several hours when this occurs, after which time the brown color of the glycogen reaction will have disappeared and the blue color of the starch reaction remains.

(2) Testing on solid media. One or more strains are inoculated onto agar medium in petri dishes. The cultures are incubated for 1–2 weeks, then flooded with dilute Lugol's iodine, and inspected for formation of a blue to green color.

Media

(1) Medium I (Lodder and Kreger-van Rij 1952), ammonium sulfate–glucose agar. Dissolve 2 g of ammonium sulfate, 2 g of potassium dihydrogen phosphate, 1 g of magnesium sulfate heptahydrate, and 20 g of glucose in 1 liter of demineralized water. Adjust the pH to 4.5. Dissolve 40 g of agar in 1 liter of demineralized water. The two portions of the media are autoclaved at 121°C for 15 min. They are put together aseptically and 20 ml of either a 20% solution of yeast extract or a 100-fold concentrated vitamin solution are added. The two solutions are mixed gently while the agar is still molten and dispensed aseptically into petri dishes.

(2) Medium II. Dissolve 10 g of glucose, 6.7 g of Bacto Yeast Nitrogen Base, and 15 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min.
(3) Lugol's iodine solution (Gram's modification). Dissolve 1 g of iodine and 2 g of potassium iodide in 300 ml of demineralized water.

3.3.1.9. Hydrolysis of urea: Seeliger (1956) tested the hydrolysis of urea by some ascogenous and anascogenous species on Christensen's urea agar. Hydrolysis is generally absent in ascogenous species, whereas it is marked in the basidiomycetous genera such as *Cryptococcus* and *Rhodotorula* (Abadie 1967, Hagler and Ahearn 1981). The ascogenous yeasts *Schizosaccharomyces pombe*, *Yarrowia lipolytica* and *Lipomyces* species are exceptions. Booth and Vishniac (1987) established the presence of urease and, probably, its role as the major enzyme concerned in the utilization of urea by yeasts. Urease catalyses the hydrolysis of urea, giving ammonia and carbamate which, itself, hydrolyses to form carbonic acid and another molecule of ammonia (Andrews et al. 1984). The overall reaction leads to an increase in pH. The test can be done on solid medium (Seeliger 1956) or in liquid medium (Barnett et al. 1990).

3.3.1.9.1. Testing on solid medium. *Method*. Cells from a young culture are inoculated onto a slant of Christensen's urea agar (Christensen 1946) and a control tube of the basal medium without urea and incubated at 25°C. The cultures are inspected daily for up to 4 days and the result is recorded as positive when a deep pink color develops in the tube of test medium but not the control.

Medium. Christensen's urea agar. Dissolve 1 g of peptone, 5 g of sodium chloride, 2 g of dihydrogen phosphate, and 12 µg of phenol red in 1 liter of demineralized water and adjust the pH to 6.8. Add and dissolve 20 g of agar and dispense 4.5-ml amounts into 16 mm diameter plugged glass tubes. Autoclave at 121°C for 15 min, then add 0.5 ml of a filter-sterilized 20% solution of urea. After mixing, slant the tubes and allow the agar to set. This medium is available in powdered form commercially.

3.3.1.9.2. Testing in liquid medium. *Method*. Difco Bacto Urea R Broth is dissolved in sterile demineralized water and amounts of 0.5 ml are dispensed into tubes aseptically. These tubes can be stored in a freezer for up to 6 months. A loopful of cells from a one- or two-day-old culture is suspended in the broth and incubated at 37°C (irrespective of whether the yeast can grow at this temperature). The tubes are examined every half hour for a change of color to red, which indicates hydrolysis of urea. Many yeasts which give a positive result produce the color change within half an hour, most do so within two hours and all within four hours.

3.3.1.10. Splitting of fat: Lipase activity has been reported in several species, including *Yarrowia lipolytica, Candida rugosa, Zygoascus hellenicus*, and *Trichosporon pullulans*. Several methods have been proposed for detecting lipolytic activity, although this property is employed mainly as a confirmatory test. These methods use substrates such as tributyrin, olive oil, tallow, Tween 40, 60, and 80, as well as partial glyceryl esters of higher fatty acids (Bulder 1955, Sierra 1957, Tuynenburg-Muys and Willemse 1965). Tuynenburg-Muys and Willemse compared the various methods and concluded that for routine purposes none was better than that developed by Eijkman (1901). They did, however, recommend the use of partial glycerol esters of higher fatty acids, which have a higher melting point than beef tallow.

Method. Melt and filter fresh beef suet, then sterilize the tallow by autoclaving it at 121°C for 15 min. Pipette approximately 0.5 ml of molten fat into a slightly warmed sterile petri dish and then tilt it from side to side so that the fat spreads evenly over the bottom. Remove any surplus fat with a pipette. Put the dish in a refrigerator for an hour or two to allow the fat to harden. Melt 18–20 ml of either YM agar (p. 79) or Gorodkowa agar (p. 87) and let it cool to about 40°C, pour over the fat and allow to set. The plates are inoculated by streaking the test strains across the plate, cutting slightly into the agar medium with the needle, and incubated at 25°C. The plates are inspected daily for the formation of an opaque zone, formed by calcium salts of the liberated fatty acids, which is usually apparent along the streak within a week.

3.3.1.11. Cycloheximide resistance: Whiffen (1948) was the first to report that yeasts varied in their sensitivity to the antibiotic cycloheximide (Acti-dione). Her results, based on the study of 22 species, indicated that yeasts could be divided into 3 categories:
(1) markedly sensitive, inhibited by 1 µg/ml, e.g., *Saccharomyces cerevisiae*;
(2) moderately sensitive, inhibited by 25 µg/ml, e.g., *Schizosaccharomyces pombe*;
(3) tolerant, not inhibited by concentrations as high as 1000 µg/ml, e.g., *Kluyveromyces lactis*.

Method. The test is done in liquid Bacto Yeast Nitrogen Base with D-glucose, as for the assimilation tests, with cycloheximide added to give a final concentration of either 0.1% or 0.01% (w/v). The results are read and scored in the same way as for the carbon assimilation tests (p. 91).

3.3.1.12. Tolerance of 1% of acetic acid: Sand (1973) and Pitt (1974) showed that yeasts could be distinguished on the basis of their resistance to benzoic and acetic acids. A test was described by Yarrow (1984d) for growth on a medium containing 1% acetic acid to discriminate *Zygosaccharomyces rouxii* from *Z. bailii*, and *Z. bisporus*.

Method. A 4-mm loopful of the same cell suspension as used to inoculate the assimilation tests is either spotted onto or streaked across an agar plate (several strains can be tested in one petri dish). The plates are incubated at 25°C and examined after 3 and 6 days for the development of colonies.

Medium. Acetic-acid (1%) agar. Dissolve 10 g of glucose, 1 g of tryptone, 1 g of powdered yeast extract, and 2 g of agar in 100 ml of demineralized water and autoclave at 121°C for 15 min. Cool the sterilized medium to between 45°C and 50°C, add 1 ml of glacial acetic acid, mix rapidly and pour into petri dishes.

3.3.1.13. Gelatin liquefaction: The ability to liquefy gelatin is of limited diagnostic value because few yeasts are strongly proteolytic. This property is used as a confirmatory test for species that liquefy gelatin rapidly and completely, within about a week.

Method. Stelling-Dekker (1931), Lodder (1934), and Lodder and Kreger-van Rij (1952) used a medium containing malt extract and 20% gelatin, whereas Wickerham (1951) preferred to use a synthetic medium containing 10% gelatin. The use of the synthetic medium is preferred for reasons of standardization. When the test is done in tubes, each tube is inoculated with the same amount of inoculum as each tube of assimilation medium. When done on plates, the surface of the medium is inoculated with a needle or a multi-point inoculator. The cultures are examined regularly for up to 3 weeks for signs of liquefaction.

Medium. Dissolve 100 g of gelatin, 5 g of glucose, and 6.7 g of Bacto Yeast Nitrogen Base in 1 liter of demineralized water, dispense into either tubes or bottles (from which the medium can be poured into petri dishes when required). Sterilize by autoclaving at 121°C for 15 min. The medium in tubes is allowed to gel with the tubes in a vertical position.

3.3.1.14. Diazonium Blue B color reaction: Van der Walt and Hopsu-Havu (1976) studied 70 yeast strains representing 26 ascogenous and 4 basidiomycetous genera and reported that all the strains of basidiomycetous yeasts, as well as those anamorphs which had cell walls with a fine structure typical of basidiomycetes, gave a dark-red color reaction when a buffered solution of Diazonium Blue B (DBB) was applied to the cultures. Ascogenous yeasts did not give this reaction.

The mechanism of the reaction has not yet been elucidated. Nevertheless, this test is employed to determine whether an asexual yeast belongs to a basidiomycetous or an ascomycetous genus. The synonymy in the chemical nomenclature of the reagent and related compounds has been discussed by Barnett et al. (1990).

3.3.1.14.1. Reaction on solid media. *Method*. The strains being tested are cultivated on YM agar. Van der Walt and Hopsu-Havu (1976) incubated the cultures for 3 weeks, however, Hagler and Ahearn (1981) reduced this period to 5–7 days followed by 16 h at 55–60°C. Hagler and Mendonça-Hagler (1991) reduced the time needed for this test even further by incubating the cultures on carbon base–urea agar at 25°C for 3 days followed by incubation overnight at 55°C. The plates are allowed to cool to room temperature after incubation before the cultures are tested. One or two drops of freshly prepared chilled DBB reagent are applied to the surface of each colony, taking care to ensure good contact between the reagent and the yeast. The result is recorded as positive if a dark-red to violet-red color develops within 1–2 min at room temperature.

Media and reagent.

(1) YM agar, see p. 79.

(2) Carbon base–urea agar. Dissolve 11.7 g of Bacto Yeast Carbon Base, 0.2 g of acid fuchsin, and 20 g of agar in 900 ml of demineralized water. Sterilize by autoclaving at 121°C for 15 min. Add 100 ml of a filter-sterilized 20% solution of urea to the molten medium, mix and dispense into either tubes or petri dishes.

(3) DBB reagent. The DBB reagent is prepared by dissolving 15 mg of Diazonium Blue B salt (Brentamine Blue B, ICI plc; Fast Blue Salt B, Farbwerke Hoechst AG) in 15 ml of chilled 0.25 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.0. The salt is unstable under warm and damp conditions, but 15-mg portions of the dye can be stored in sealed tubes at 4°C. The freshly prepared reagent is kept in an ice bath and used before it turns dark yellow (within about 30 min).

3.3.1.14.2. Reaction in liquid medium. *Method*. The strains to be tested are cultivated in 5 ml of nitrogen base with 0.5% glucose (pH 7.0) for 3 days with agitation at a suitable temperature. Slow-growing strains may have to be cultivated for longer to obtain sufficient material for the test. The cells are collected in a centrifuge and the supernatant is poured off. The cells are then suspended in 0.5 ml of 0.05 N KOH and the tube is placed in boiling water for 10 min. The suspension is cooled to ambient temperature and mixed well with 2.5 ml of 95% ethanol. The cells are sedimented by centrifuging briefly and the supernatant is poured away. The cells are suspended in 0.3 ml of DBB reagent. The test is scored positive if a violet color develops within a few seconds. The color is retained by the cells for several hours if 0.1 ml of 95% ethanol is added immediately after the color has formed. Some workers scale this test down and use one fifth of the amounts of medium and reagents given above.

3.3.1.15. Canavanine–Glycine–Bromothymol blue (CGB) agar for identifying the varieties of *Filobasidiella neoformans* (*Cryptococcus neoformans*): *Filobasidiella neoformans* variety *neoformans* can be distinguished from the variety *bacillispora* within 2–5 days by incubating cultures on CGB agar at 25°C. Isolates of the variety *bacillispora* hydrolyze glycine and are resistant to L-canavanine. The pH of the medium rises and the color of the bromothymol blue indicator turns to blue when the glycine is hydrolyzed. Most isolates of the variety *neoformans* do not hydrolyze glycine, and the few which do are sensitive to the canavanine so the medium remains greenish-yellow in color (Kwon-Chung et al. 1982b, Min and Kwon-Chung 1986).

Method. A loopful of cells from a young culture is streaked onto CGB agar in either a tube or a petri dish and incubated at 25°C for up to 5 days. The color of the

medium does not change if the strain is *Filobasidiella neoformans* var. *neoformans*, but turns blue if the strain is the variety *bacillispora*.

Medium. Canavanine–glycine–bromothymol blue (CGB) agar. The complete medium is prepared by cooling 900 ml of bromothymol agar (c, below) to about 55°C and adding 100 ml of stock solution A (a, below).

(a) Stock solution A. Dissolve 300 mg of L-canavanine sulfate, 100 g of glycine, 10 g of potassium dihydrogen phosphate, 10 g of magnesium sulfate heptahydrate, and either 10 drops of Bejectal with vitamin C (Abbott Laboratories, Chicago, IL, USA) or 10 mg of thiamine hydrochloride in 1 liter of demineralized water, adjust the pH to 5.6 and filter sterilize.
(b) Bromothymol blue solution. Dissolve 0.4 g of sodium bromothymol blue in 100 ml of demineralized water and filter sterilize.
(c) Bromothymol blue agar. Dissolve 20 g of agar in 880 ml of demineralized water and add 20 ml of bromothymol blue solution. Sterilize by autoclaving at 121°C.

3.3.1.16. Melanin synthesis on DOPA medium: The formation of melanin-like pigments from various *ortho*- and *para*-diphenols is used for rapid presumptive identification of *Filobasidiella neoformans*. A variety of substrates may be used: extracts of the seeds of *Guizotia abyssinica* (Niger seed), catechol, chlorogenic acid, protocatechuic acid, norepinephrine, dopamine, hydroxymetanilamine, dihydroxymetanilamide, and dihydroxyphenylalanine (DOPA) (Staib 1962, Chaskes and Tyndall 1975, 1978, Nurudeen and Ahearn 1979).

Method. Cells from a young culture are streaked onto DOPA agar in either a tube or a petri dish and incubated at a temperature of 20–30°C for up to 72 h. Colonies of *Filobasidiella neoformans* are black on this medium.

Medium

(1) DOPA solution. Dissolve 0.04 g of dihydroxyphenylalanine (DOPA), 1 g of asparagine, 1 g of L-glutamine, and 1 g of glycine in 200 ml of demineralized water. Adjust the pH to 5.5 with 1 M potassium hydrogen phosphate (about 2.5 ml is needed) and filter sterilize the solution.
(2) Basal medium. Dissolve 4 g of potassium dihydrogen phosphate, 2.5 g of magnesium sulfate heptahydrate, 10 mg of thiamine hydrochloride, 20 µg of biotin, 0.5 g of glucose, and 25 g of agar in 800 ml of demineralized water. Adjust the pH to 5.5 with approximately 7 ml of 1 M potassium hydrogen phosphate and sterilize by autoclaving at 121°C for 15 min. Cool the agar medium to 55°C and mix with the DOPA solution before pouring into petri dishes or test tubes. The complete medium should be stored at 4°C and used within one week of preparation.

3.3.1.17. Tetrazolium indicator medium (TTC medium): Media containing tetrazolium salts are used in some medical laboratories as an aid in isolating and presumptively identifying some species (Pagano et al. 1957, Mendel et al. 1960, Yamane and Saitoh 1985). *Candida tropicalis* produces dark red to maroon colonies owing to the reduction of the tetrazolium salt, whereas yeasts which are incapable of reducing the tetrazolium salt, such as *Candida albicans*, produce pale pink to whitish colonies.

Method. Cells are either streaked or spotted onto the agar. The plate is incubated for 24–48 h and then inspected for colored colonies.

Medium

(1) TTC stock solution. Dissolve 1 g of 2,3,5-triphenyltetrazolium chloride in 10 ml of demineralized water. Sterilize by filtration and store at 4°C in a dark glass bottle.
(2) Basal medium. Dissolve 10 g of mycological peptone, 10 g of glucose, and 15 g of agar in 1 liter of demineralized water. Sterilize by autoclaving at 121°C for 15 min. Melt the agar just before use and cool to 45–50°C, then add 0.1 ml of the stock TTC solution, to give a final concentration of 100 µg per ml, and pour into petri dishes. The final pH of the medium is 7.0.

3.3.1.18. Staining nuclei: Although several methods have been recommended for staining the nuclei of yeast cells, none surpass the Feulgen–Giemsa technique of Ganesan and Swaminathan (1958) as modified by Robinow (1961). This method, if followed carefully, gives good preparations and has been applied for chromosomal counts. The method involves the hydrolysis of albumin-mounted cells in 1 N HCl at 60°C, followed by washing in a buffer and the application of the Giemsa stain. It should be kept in mind that not only is the time allowed for hydrolysis critical but also that it varies for different yeasts. It is therefore necessary to determine the best time to allow for hydrolysis. The publications of Robinow (1961) and McCully and Robinow (1972a,b) should be consulted for further details.

4. List of observations and tests included in the standard descriptions

Table 14
Composition of chemically defined media[a]

Formula ingredients per liter	Yeast morphology agar	Yeast nitrogen base for carbon assimilation tests	Yeast carbon base for nitrogen assimilation tests	Vitamin-free yeast base for vitamin requirement test
Nitrogen sources				
Ammonium sulfate	3.5 g	5 g	none	5 g
Asparagine	1.5 g	none	none	none
Carbon source				
Glucose (dextrose)	10 g	none	10 g	10 g
Amino acids				
L-Histidine monohydrochloride	10 mg	10 mg	1 mg	10 mg
DL-Methionine	20 mg	20 mg	2 mg	20 mg
DL-Tryptophan	20 mg	20 mg	2 mg	20 mg
Vitamins				
Biotin	20 μg	20 μg	20 μg	none
Calcium pantothenate	2000 μg	2000 μg	2000 μg	none
Folic acid	2 μg	2 μg	2 μg	none
Inositol	10 000 μg	10 000 μg	10 000 μg	none
Niacin	400 μg	400 μg	400 μg	none
p-Aminobenzoic acid	200 μg	200 μg	200 μg	none
Pyridoxine hydrochloride	400 μg	400 μg	400 μg	none
Riboflavin	200 μg	200 μg	200 μg	none
Thiamine hydrochloride	400 μg	400 μg	400 μg	none
Compounds supplying trace elements				
Boric acid	500 μg	500 μg	500 μg	500 μg
Copper sulfate	40μg	40 μg	40 μg	40 μg
Potassium iodide	100 μg	100μg	100 μg	100 μg
Ferric chloride	200 μg	200 μg	200 μg	200 μg
Manganese sulfate	400 μg	400 μg	400 μg	400 μg
Sodium molybdate	200 μg	200 μg	200 μg	200 μg
Zinc sulfate	400 μg	400 μg	400 μg	400 μg
Salts				
Potassium phosphate, monobasic	0.85 g	0.85 g	0.85 g	0.85 g
Potassium phosphate, dibasic	0.15 g	0.15 g	0.15 g	0.15 g
Magnesium sulfate	0.5 g	0.5 g	0.5 g	0.5 g
Sodium chloride	0.1 g	0.1 g	0.1 g	0.1 g
Calcium chloride	0.1 g	0.1 g	0.1 g	0.1 g
Agar	18 g	none	none	none
Amount of final medium from 100 g of dehydrated medium (liters)	2.8	14.9	8.5	5.9
Amount of dehydrated medium per liter of finished medium	35.0 g	6.7 g	11.7 g	16.7 g

[a] These media are marketed by the Difco Laboratories of Detroit, Michigan, USA, in dehydrated form; they are based on the formulae published by Wickerham (1951). They have been fortified with greater amounts of biotin, inositol and calcium pantothenate. The pH has been raised from 5.2 to 5.6 by the phosphates listed in the table.

5. List of media, reagents and stains

5.1. Media

See Table 14 for the composition of chemically defined media.

5.2. Reagents

5.3. Stains

Chapter 12

Identification of coenzyme Q (ubiquinone) homologs

Y. Yamada

Contents

1. Introduction

The yeasts and yeastlike fungi have coenzyme Q or ubiquinone as a respiratory component. The coenzyme Q homologs of these organisms are recognized to be Q-5 through Q-10 (Yamada and Kondo 1973, Yamada et al. 1976b, 1981). The chemical structure of the isoprenoid quinone is designated as 2, 3-dimethoxy-5-methyl-6-multiprenyl^n-1, 4-benzoquinone (Fig. 32). A dihydrogenated isoprenoid side-chain coenzyme Q homolog, Q-10(H_2) was found in basidiomycetous yeasts (Yamada et al. 1973c, Nakase and Suzuki 1986d). Of the four procedures described below, the usage of high performance liquid chromatography gives a qualitative as well as quantitative analysis in the identification of isoprenoid quinones. The coenzyme Q homologs are useful for classifying yeasts and yeastlike fungi at the generic level (Yamada and Kondo 1972b, Yamada et al. 1976a).

Fig. 32. Diagrammatic representation of coenzyme Q. The coenzyme Q homologs are expressed as Q-*n*, with *n* denoting a specified number of isoprene units in a side chain, e.g., Q-6, Q-10. If there are 2 hydrogen atoms saturating the isoprene units in the side chain the formula becomes Q-*n*(H_2), e.g., Q-10(H_2).

2. Methods

2.1. Growth conditions and extraction of coenzyme Q

The yeasts and yeastlike fungi are aerobically grown in a glucose/peptone/yeast extract medium (2/0.4/0.3%, w/v, 500 ml) on a rotary shaker for 24–72 h (Yamada and Kondo 1973, Yamada et al. 1989c). The cells harvested by centrifugation are suspended in 150 ml of water/methanol (1:2, v/v), containing 20 g NaOH and 5 g pyrogallol. The hydrolysis of the yeast cells is performed by heating for 30 min in a 95°C water bath (or a mantle heater). After cooling with tap water for 25 min, the coenzyme Q is extracted by vigorous shaking with 100 ml of hexane, followed by centrifugation for 2 min at 3000 rpm. This procedure is repeated two times. The pooled hexane extracts are shaken three times with 30 ml of water in a separatory funnel for removal of the NaOH. After evaporation to dryness under reduced pressure, the residue is treated with 30 ml of acetone. The yellow-colored acetone solution is concentrated to a small volume (1 ml) *in vacuo.*

2.2. Purification of coenzyme Q

The purification of coenzyme Q is carried out by preparative thin-layer chromatography (0.5 mm silica gel $60F_{254}$ layers on 20×20 cm glass plates, E. Merck) using benzene for development. A yellow band (Rf 0.3–0.5), corresponding to the yellow spot of a reference standard (Q-6 and/or Q-10), also visualized as a dark band under a short-wave UV light (wave length, 254 nm), is scraped off. The yellow silica gel powder is transferred to a filter paper in a funnel, and the yellow substance is extracted with a small volume of acetone (2 ml). The yellow solution is concentrated to a very small volume (150 μl) *in vacuo.* The isoprenoid quinones can be crystallized in ethanol at –20°C.

2.3. Identification of coenzyme Q homologs

2.3.1. Reversed-phase paper chromatography: A filter paper strip (12×40 cm, Whatman No. 1 or Toyo No. 50) is impregnated with 3.0% silicon oil (w/w, KF-54, Shin'etsu Chem. Co.) in chloroform (Yamada and Kondo 1973, Yamada et al. 1989c). Three samples can be spotted inside on the paper strip using the known reference standards (Q-6 through Q-10) on both sides. The paper strip is developed with ethanol/ethyl acetate/water (5:3:1, v/v/v). Likewise, another filter paper strip is impregnated with 2.5% white vaseline (w/w, U.S. Pharm.) in toluene. In the latter case, the solvent system is *N*, *N*-dimethylformamide/water (97:3, v/v). After developing and drying the paper strips, the coenzyme Q homologs are located as yellowish-brown spots on the chromatograms by oxidation with 0.3% $KMnO_4$ (w/v) in water. The remaining $KMnO_4$ is eluted from the paper strips with tap water.

2.3.2. Reversed-phase thin-layer chromatography: The samples are spotted on a reversed-phase thin-layer plate (HPTLC RP-18F_{254S}, 10×10 cm, 0.2 mm, E. Merck), which is then developed with acetone/acetonitrile (4:1, v/v) (Collins and Jones 1981). The spots of the coenzyme Q homologs are visualized under a short-wave UV light (wave length, 254 nm) or by spraying 50% H_2SO_4 (v/v), followed by heating the plate in an oven at 120–150°C for 2–5 min.

2.3.3. High performance liquid chromatography: The separation of the coenzyme Q homologs is performed on a Novapak C_{18} column (3.9×150 mm, Waters). Other usable columns are: Zorbax ODS (4.6×150 mm, Du Pont); Cosmosil 5C_{18} (4.6×150 mm, Nacalai); μ Bondapak C_{18} (3.9×300 mm, Waters). The mobile phase is methanol/isopropanol (7:3 and/or 1:1, v/v), and the flow rate is 1.0 ml/min (or 1.5–2.0 ml/min) (Collins and Jones 1981, Tamaoka et al. 1983, Billon-Grand 1985, Nakase and Suzuki 1985a). Detection is done at 275 nm. The sample peaks are compared with those of known reference standards (Q-6 through Q-10).

2.3.4. Mass spectroscopy: The samples are vaporized at the ion source with a direct inlet system operating at 140–170°C (Yamada et al. 1969, Yamada and Kondo 1973). Mass spectra of the coenzyme Q homologs show intense fragment ion peaks at *m/z* 197 and 235. Besides these characteristic ion peaks, several small fragment ion peaks are found at M-15 and M-69, M-69-68, M-69-68-68 and so on, which indicate the expulsion of a methyl group and the successive loss of isoprene units in the side chain, respectively. The molecular ions (M^+) are: at *m/z* 522 (Q-5), 590 (Q-6), 658 (Q-7), 726 (Q-8), 794 (Q-9) and 862 (Q-10). The dihydrogenated isoprenoid side-chain ubiquinone 10, Q-10(H_2) has the molecular ion peak at *m/z* 864. These molecular ion peaks are often observed at $M^+ + 2$, which indicates the presence of reduced coenzyme Q.

Chapter 13

Analysis of carbohydrate composition of cell walls and extracellular carbohydrates

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Contents

1. Introduction

Cell wall composition is a useful marker to indicate taxonomic and phylogenetic affiliations among fungi (Bartnicki-Garcia 1968, 1970, Weijman and Golubev 1987, Dörfler 1990, Prillinger et al. 1990a,b, 1991a,b, 1993). Bartnicki-Garcia (1970) divided the fungi into eight groups using combinations of the two most dominant cell wall carbohydrates present. Weijman and Golubev (1987) distinguished six categories of yeasts and yeastlike fungi using qualitative and semi-quantitative analyses of cell walls. The following groups were recognized:

(1) Saccharomycetes (Endomycetes) pro parte (e.g., Saccharomycetaceae), characterized by high mannan, low chitin content, and absence of galactose;
(2) the remaining Saccharomycetes (Endomycetes) (e.g., Dipodascaceae, Nadsoniaceae) have high mannan content, intermediate chitin content, and contain galactose;
(3) the "red yeasts" (Sporobolomycetaceae, Sporidiobolaceae): variable mannan content, intermediate chitin content, and contain fucose or rhamnose;
(4) the smuts (Ustilaginales): low mannan content, but high chitin content;
(5) the remaining basidiomycetes (i.e., Filobasidiaceae and Cryptococcaceae): low mannan content, but with chitin and xylose present; and
(6) the Euascomycetes: low mannan content, but contain chitin and rhamnose.

Prillinger et al. (1993) differentiated seven cell wall types among the yeasts and yeastlike fungi using both quantitative and qualitative analyses. Their typology is a refinement of that of Weijman and Golubev (1987). Three cell wall types occur within the ascomycetous yeasts:

(1) *Saccharomyces*-type with mannose and glucose present;
(2) *Schizosaccharomyces*-type with galactose, glucose and mannose present;
(3) *Protomyces*-type with glucose predominant, rhamnose and mannose present, and galactose commonly present.

The presence of glucose, mannose and galactose is found in different orders of Ascomycetes (Saccharomycetales, Schizosaccharomycetales, *Saitoella*, and different orders of filamentous Ascomycetes) indicating that the phylogenetic value of galactose is rather low (Prillinger et al. 1994). Four cell wall types occur within the basidiomycetous yeasts:

(1) *Microbotryum*-type with mannose dominant, glucose present, fucose usually present, and rhamnose sometimes present;
(2) *Ustilago*-type with glucose dominant, and mannose and galactose present;
(3) *Dacrymyces*-type with xylose present, and glucose and mannose present in equal amounts, traces of galactose may be present, but extracellular amyloid compounds are usually absent;
(4) *Tremella*-type with glucose predominant, xylose, mannose, and galactose present, and extracellular amyloid compounds are often present.

The latter four types agree with the cell wall typology of the Basidiomycetes given by Dörfler (1990). From these data it is apparent that cell wall biochemistry is an important tool in the taxonomy and phylogeny of yeasts and yeastlike organisms.

Four main methods have been applied to analyze the carbohydrate composition of the yeast cell wall:

(1) Gas chromatographic analysis of acid hydrolyzates of whole cells with derivatization using capillary columns (Weijman 1976, Weijman and Golubev 1987) or packed columns (Sugiyama et al. 1985);
(2) gas chromatographic analysis of acid hydrolyzates of purified cell walls with derivatization (Dörfler 1990, Prillinger et al. 1990a,b, 1991a,b, 1993);
(3) high performance liquid chromatographic (HPLC) analysis of acid hydrolyzates of whole cells without derivatization (Suzuki and Nakase 1988a); and
(4) high performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) of cell wall neutral sugars without derivatization (Prillinger et al. 1993).

Weijman's method is summarized as follows: cells are hydrolyzed with 1 N HCl for 12 h at 100°C. During hydrolysis monomers are formed. Neutral polysaccharides are hydrolyzed completely at low concentrations of HCl (1 N), whereas chitin is converted to glucosamine at high concentrations of HCl (5 N). After hydrolysis,

the solubilized components are trimethylsilylated (TMS) prior to gas–liquid chromatography. Sugiyama's method differs in that dried cells are hydrolyzed in 2.5 N trifluoroacetic acid (TFA) at 100°C for 15 h, followed by reduction of the neutral sugars to their corresponding alditols by borohydride and acetylation of the alditol derivatives by acetic acid anhydride. The final residues are trifluoroacetylated, followed by gas chromatographic analysis. In Prillinger's method cell walls are isolated and purified before further processing. In order to accurately detect xylose, Suzuki and Nakase (1988a) developed a method using HPLC analysis of whole-cell hydrolyzates without derivatization. In brief, whole cells are hydrolyzed with TFA and directly analyzed using HPLC.

2. Methods

2.1. Analysis of whole-cell hydrolyzates using trimethyl-silylation (Weijman 1976, Weijman and Golubev 1987)

Yeast cells are grown in 100 ml yeast extract–peptone–glucose (YPG) broth in 300 ml Erlenmeyer flasks on a rotary shaker at 150 rpm and 24°C (psychrophilic species at 17°C). After 5–7 days, the cells are harvested by centrifugation (9000g), washed with 0.9% NaCl and washed again with deionized water. The pellet is freeze-dried and powdered. Fifteen mg dried cells are hydrolyzed in 6 ml 1 N HCl or 5 N HCl under nitrogen in glass tubes with a screwcap for 12 h at 100°C in a sandbath. To detect xylose, the cells are hydrolyzed with 2 N trifluoroacetic acid for 3 h at 100°C. After cooling, the hydrolyzates are filtered through Whatman No. 1 filter paper and 1 ml of the filtrate is dried in a rotary evaporator. An additional 100 μl Tri-Sil (Pierce) is used to silylate the sample. The reaction mixture is vigorously shaken and allowed to stand for 15 min. One μl is injected into the gas chromatograph-mass spectrometer (GC-MS), which is equipped with a wall coated open tubular (WCOT) capillary column of 25 meters, coated with CP Sil 5CB with a film thickness of 0.13 μm and an inside diameter of 0.32 mm. The column is programmed from 125 to 175°C with a rate of 10°C min^{-1} and an isothermal period of 5 min. Helium is used as the carrier gas at a flow rate of 30 ml min^{-1}. Electron Impact (EI) at 70 eV is used for ionization and a quadrupole serves as a massfilter.

2.2. Analysis of whole-cell hydrolyzates using trifluoroacetic acid (TFA) and reduction of sugars to their alditol derivatives (Sugiyama et al. 1985)

Yeast cells are grown in liquid Wickerham's basal nitrogen medium supplemented with 15 ml 1% glucose at 25°C for 3–5 days on a test tube shaker. Cells are harvested by centrifugation, and washed with deionized water. The pellet is freeze-dried and powdered. About 30 mg of the dry cell powder is hydrolyzed in 5 ml 2.5 N trifluoroacetic acid (TFA) at 100°C for 15 h in a sealed tube. The remaining acid is removed by drying over a rotary evaporator. Fifty mg of sodium borohydride in 10 ml distilled water is added to the residue. The reaction mixture is allowed to stand overnight to reduce the sugars to alditols. Excess sodium borohydride is removed by adding dropwise 5% hydrochloric acid in methanol and by evaporating to dryness. Insoluble material and low-polar materials are removed by membrane filtration (0.45 μm, Gelman Sciences, Inc., Ann Arbor, MI, USA), followed by reversed-phase chromatography (Sep-Pak C, Waters Associates, Milford, MA, USA). After drying, 2 ml of methanol are added. The solution is dried in a rotary evaporator to remove the borate complex. This step is repeated several times. To 10 mg of the residue, 0.1 ml of trifluoroacetic anhydride and 0.1 ml of *N*-methylbis-trifluoroacetamide are added. The reaction mixture is kept in a sealed tube and left overnight. One to 2.5 μl of the sample is injected in a gas chromatograph equipped with a hydrogen flame ionization detector. The U-shaped glass column (4 m × 3 mm i.d.) is packed with Chromosorb W (HP) 80–100 mesh coated with 2% silicone OV-105, 800 mesh. Nitrogen is used as the carrier gas at a flow rate of 35 ml min^{-1}. The column temperature is 140°C, and the injector temperature is 150°C. Carbohydrates are identified on the basis of sample coincidence with the relative retention times for the trifluoroacetyl derivatives of the neutral monosaccharide standards.

2.3. Analysis of whole cell hydrolyzates without derivatization using HPLC (Suzuki and Nakase 1988a)

Yeast cells are grown in a 500-ml Erlenmeyer flask containing 200 ml YM broth supplemented with 2% glucose on a rotary shaker at 150 rpm and 25°C (17°C for psychrophilic species). After 4–5 days the cells are harvested by centrifugation (5000 rpm) and washed twice with deionized water. Fifty to 100 mg of acetone dried cells are suspended in 2 ml 2 M trifluoroacetic acid (TFA) in a test tube (13 × 100 mm) with a teflon-sealed screw cap, and kept at 100°C for 3 h in a metal block bath. After cooling, the hydrolyzate is filtered through paper and evaporated to dryness. The residue is dissolved in 0.5 ml water, neutralized with small amounts of Amberlite IRA 410 (OH form), filtered with a disposable filter unit (e.g., Shodex DT ED-13), and then subjected to HPLC. HPLC was performed using two different column systems, namely:

(1) with water (HPLC grade) as the mobile phase at a flow rate of 0.8 ml min^{-1} at 80°C; and
(2) with acetonitrile-water (80:20, v/v, HPLC grade) as the mobile phase at a flow rate of 0.8 ml min^{-1} at 75°C.

A refractive index detector is used to detect the carbohydrates. Neutral sugars and sugar alcohols are identified by comparing their retention times with those of standard neutral sugars and sugar alcohols.

2.4. Isolation and purification of cell walls (Prillinger et al. 1993)

Yeast cells are grown in 5×100 ml YPG broth on a rotary shaker at 150 rpm for 3–5 days, harvested by centrifugation (3000 rpm), washed with deionized water until the supernatant is clear, and frozen at –20°C until further use.

For disruption, cells are suspended in distilled water (1:1, v/v), and disrupted in a French Press (20 000 PSI) until no intact yeast cells are present under the light microscope. Recently, Messner et al. (1994) have shown that disintegration of yeast cells by a Vibrogen Cell Mill (Tübingen, Germany) and 0.5 mm glass beads (yeast pellet/distilled water/glass beads = 1/1/3, w/w) is superior to the disruption with a French Press. Disrupted cells are washed with ice-cold distilled water until the supernatant is clear. To remove cytoplasmic remnants, the cell walls are thoroughly washed twice with 1% Na-desoxycholate (pH 7.8) with intensive stirring. After each Na-desoxycholate purification the cell walls are rinsed three times with distilled water. In the case of capsuled yeasts, all the capsular material, which may form a second slimy layer above the cell wall pellet, should be removed. Yeast cells without capsules (i.e., those not having a positive starch test with Lugol's solution) are lyophilized and powdered with a pestle and mortar and further processed.

2.5. High performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) of cell wall neutral sugars without derivatization (Prillinger et al. 1993)

Acid hydrolysis of purified cell walls and removal of TFA are performed according to the method of Sugiyama et al. (1985) (see above). Usually a mixture of 2 mg of powdered cell wall suspended in 2 ml 2 N TFA is hydrolyzed for 2 h at 120°C using teflon-sealed Pyrex test tubes. A standard mixture of monosaccharides containing 90 µg of each neutral sugar is treated in the same way. After blowing off the TFA, samples and standards are resolved in 10 ml distilled water. Monosaccharides are separated on a Dionex CarboPac PA-1 column (4.6×250 mm), equipped with a guard column, using a flow rate of 1 ml min^{-1} at room temperature. Elute with NaOH as follows: 10 mM NaOH for 3.9 min isocratic, followed by a step gradient to 100% deionized water for 30 min, and reequilibration to the initial conditions for 10 min. The system used for monosaccharide analysis consisted of a Dionex (Sunnyvale, CA) Gradient Pump Module GPM 2 and a Pulsed Amperometric Detector PAD 2. A Dionex Eluant Degas Module is used to sparge and pressurize the eluants with helium. Eluant 1 is 100 mM NaOH (preparation of a 50% NaOH stock solution with ultrapure distilled water), and eluant 2 is 18 MOhm deionized water. Sample injection is via a Dionex High Pressure Injection Valve equipped with a 10 µl sample loop. To ensure a carbonate free eluant, an anion trap column ATC-1 was installed before the injection valve. Detection of the separated monosaccharides is by a PAD, equipped with a gold working electrode. The following pulse potentials are used: $E_1 = 0.1$ V ($t_1 = 300$ ms); $E_2 = 0.6$ V ($t_2 = 120$ ms); $E_3 = -0.6$ V ($t_3 = 60$ ms). The response time of the PAD 2 is set to 1 s. Resulting data are integrated and plotted using Dionex A1-450 software.

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Chapter 14

Determination of ethanol production

Ann Vaughan-Martini and Alessandro Martini

Contents

1. Introduction

Several yeast species are capable of forming ethanol by fermentation of monosaccharides and oligosaccharides. The quantitative expression of this property is, however, quite variable among fermenting yeasts, ranging from less than 1–2% (w/v) to 14% and higher in some strains of the *sensu stricto* group of the genus *Saccharomyces* (p. 358).

Tolerance to concentrations of ethyl alcohol higher than 12–13% (w/v) is a technological character highly appreciated in the case of yeasts utilized as fermentation starters in the wine industry. Screening surveys for this property (fermenting power) have been done on a large number of strains isolated from grapes, fermenting grape musts and wines (Kunkee and Goswell 1977) by using a simple method that allows a rough evaluation of ethanol yields from the gravimetric estimation of the CO_2 formed in a liquid fermentation medium containing an excess of glucose (24% w/v).

The test is carried out at 25°C in Erlenmeyer flasks plugged with a special glass device (Müller valve) that causes the CO_2 produced by fermentation to pass through concentrated sulfuric acid (Fig. 33). This acts as a water trap to eliminate weight loss as water vapor.

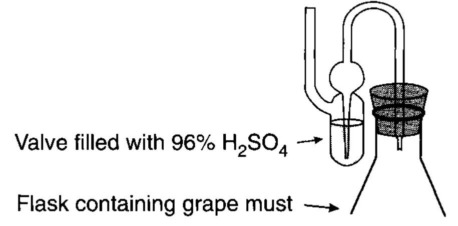


Fig. 33. Müller valve assembly for determining ethanol production from fermentation.

2. Method

Cells from a 24 h preculture grown with agitation (150 rpm) at 25°C are centrifuged, washed twice and re-suspended in sterile, deionized water. 200-ml Erlenmeyer flasks with 100 ml of synthetic medium are inoculated to a final concentration of 5×10^6 cells per ml and closed with the Müller valve previously filled with sulfuric acid. A zero time weight value is taken before incubating in static culture at 25°C. Flasks are weighed daily until values are stationary. Relative ethanol production (as volume percent) is calculated by multiplying the weight loss (as grams of CO_2) by 1.25.

3. Media

Although grape must adjusted to 24% glucose concentration has been used by some authors (Capriotti 1954, Rosini et al. 1982b), more reliable results are obtained with the following synthetic medium (Ciani and Rosini 1987): 13.7 g/l Bacto Yeast Nitrogen Base (Difco, Detroit, MI, USA); glucose 24% (w/v), pH adjusted to 3.0. Sterilization is preferably done by Tyndalization which involves the exposure of the medium to steam on three consecutive days. The medium is steamed for 30 min on the first day, incubated at room temperature overnight, steamed for a further 30 min on the second day, reincubated and steamed again for 30 min on the third day. The first day's exposure kills non-spore-forming organisms, i.e., vegetative forms; subsequent incubation, provided the medium is favorable, allows germination of bacterial endospores, which are killed during the second or third steaming.

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Part Va

Classification of the ascomycetous taxa

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Chapter 15

Discussion of teleomorphic and anamorphic ascomycetous yeasts and a key to genera

C.P. Kurtzman

Contents

1. Introduction

Historically, ascomycetes have been placed in two taxonomic classes, or as subclasses in some treatments, the Hemiascomycetes and the Euascomycetes. Hemiascomycetes are characterized by asci that are not enclosed within a fruiting body (ascocarp), whereas Euascomycetes usually form asci within or upon fruiting bodies. Budding yeasts, fission yeasts and yeastlike genera such as *Ascoidea, Cephaloascus,* and *Taphrina* are assigned to the Hemiascomycetes, but taxonomic treatments of yeasts typically exclude the yeastlike taxa.

Earlier proposals for classification of the ascomycetous yeasts have been summarized by Lodder (1970) and Kreger-van Rij (1984a). Those systems were mostly based on type of vegetative cell division, ploidy and morphology of ascospores. Another aspect of classification is whether the ascomycetous yeasts represent primitive organisms, as is suggested by their appearance, or whether they are reduced forms derived from various more advanced fungal lineages. Cain (1972) proposed that yeasts represent reduced forms, citing the hat-shaped (galeate) ascospores common to *Pichia (Hansenula), Cephaloascus,* and *Ceratocystis* as evidence of an adaptation that would have occurred just once in evolutionary history. Redhead and Malloch (1977) accepted this proposal and further argued for the derived status of various yeastlike ascomycetes, which they assigned to existing families of mycelial fungi. Von Arx and van der Walt (1987) also agreed with the concept that ascospore shape is of phylogenetic significance and suggested additional relationships between yeasts and euascomycete families.

In the last few years, the system of classification for yeasts, as well as for other fungi, has been revolutionized by the introduction of phylogenetic analysis of molecular sequences. A majority of the studies have focused on rRNA gene sequences, but comparisons of other molecules have given similar results (Kurtzman 1994). Details of these studies are discussed in the chapter on rRNA/rDNA sequence comparisons as well as in many of the chapters on individual genera. These studies (Barns et al. 1991, Berbee and Taylor 1993, Hausner et al. 1992, Hendriks et al. 1992, Kurtzman 1993a,c, 1994, Kurtzman and Robnett 1994a; Nishida and Sugiyama 1993, Walker 1985a) have demonstrated the following: (1) budding yeasts and yeastlike taxa such as *Ascoidea* and *Cephaloascus* are members of the same clade and this clade is a sister group to the euascomycetes, some of which are dimorphic and have a yeast phase; (2) with few exceptions (e.g., *Eremascus*), euascomycetes form asci within or upon a fruiting body, whereas members of the yeast clade do not; (3) *Schizosaccharomyces, Taphrina, Protomyces, Saitoella* and *Pneumocystis* form a divergent group of taxa basal to the other ascomycetes; (4) ascospore shape, presence or absence of hyphae and pseudohyphae, presence or absence of budding, and most assimilation reactions are not predictive of phylogenetic relationships.

The classification system used for ascomycetous taxa in this edition of *The Yeasts, A Taxonomic Study,* is given in Table 15. The class "Archiascomycetes" was proposed by Nishida and Sugiyama (1993) for those basal taxa now represented by *Schizosaccharomyces, Taphrina, Protomyces, Saitoella* and *Pneumocystis.* Although genetic distances within this clade are uncertain, it has been divided into four orders. The Taphrinales and the Protomycetales appear closely related and may prove synonymous. Eriksson (1994) recently described the order Pneumocystidales for placement of the genus *Pneumocystis.* Prillinger et al. (1990a), noting the many differences that previously had been observed between *Saccharomyces* and *Schizosaccharomyces,* introduced the order Schizosaccharomycetales. However, Prillinger et al. (1990a) indicated a possible basidiomycetous affinity for this new order. Because of this questionable link to the basidiomycetes and the earlier uncertainty concerning the relationship of *Schizosaccharomyces* with *Dipodascus* and *Geotrichum,* the order Schizosaccharomycetales was emended by Kurtzman (1993a) to reflect its ascomycetous nature and phylogenetic scope. Eriksson et al. (1993) examined the molecular and organismal differences between *Saccharomyces* and *Schizosaccharomyces* and also proposed the order Schizosaccharomycetales. Because Prillinger et al. (1990a) first made the proposal for ordinal rank, Schizosaccharomycetales Prillinger et al.

Table 15

Classification of the ascomycetous yeasts[a]

Class	Order	Family[b]	Genus
Phylum: Ascomycota			
"Archiascomycetes"			
	Schizosaccharomycetales Prillinger, Dörfler, Laaser, Eckerlein & Lehle ex Kurtzman		
		Schizosaccharomycetaceae Beijerinck ex Klöcker	
			Schizosaccharomyces
	Taphrinales Gäumann & C.W. Dodge		
		Taphrinaceae Gäumann	
			Taphrina
			Lalaria (Anamorph of *Taphrina*)
	Protomycetales Luttrell ex D. Hawksworth & O.E. Eriksson		
		Protomycetaceae Gray	
			Protomyces
			? *Saitoella* (Anamorphic genus)
	Pneumocystidales O.E. Eriksson		
		Pneumocystidaceae O.E. Eriksson	
			Pneumocystis
Euascomycetes			
			? *Endomyces*[c,d] (*E. scopularum*)
			Oosporidium
Hemiascomycetes			
	Saccharomycetales Kudryavtsev (synonym Endomycetales Gäumann)		
		Ascoideaceae J. Schröter	
			Ascoidea
		Cephaloascaceae L.R. Batra	
			Cephaloascus
		Dipodascaceae Engler & E. Gilg	
			Dipodascus
			Galactomyces
			? *Sporopachydermia*
			? *Stephanoascus*
			? *Wickerhamiella*
			? *Yarrowia*
			? *Zygoascus*
		Endomycetaceae J. Schröter	
			? *Endomyces*[c,d] (*E. decipiens*)
			? *Helicogonium*[c]
			? *Myriogonium*[c]
			? *Phialoascus*[c]
			? *Trichomonascus*[c]
		Eremotheciaceae Kurtzman	
			Eremothecium
			? *Coccidiascus*
		Lipomycetaceae E.K. Novák & Zsolt	
			Babjevia
			Dipodascopsis
			Lipomyces
			Zygozyma
		Metschnikowiaceae T. Kamienski	
			Clavispora
			Metschnikowia
		Saccharomycetaceae G. Winter	
			Arxiozyma
			? *Citeromyces*
			? *Cyniclomyces*
			? *Debaryomyces*
			? *Dekkera*
			? *Issatchenkia*
			Kluyveromyces
			? *Lodderomyces*
			? *Pachysolen*
			? *Pichia*
			Saccharomyces
			? *Saturnispora*
			Torulaspora
			? *Williopsis*
			Zygosaccharomyces
		Saccharomycodaceae Kudryavtsev	
			? *Hanseniaspora*
			? *Nadsonia*
			Saccharomycodes
			? *Wickerhamia*
		Saccharomycopsidaceae von Arx & van der Walt	
			? *Ambrosiozyma*
			Saccharomycopsis
		Candidaceae Windisch ex van der Walt (Anamorphic)	
			Aciculoconidium
			Arxula
			Blastobotrys
			Botryozyma
			Brettanomyces
			Candida
			Geotrichum
			Kloeckera
			Myxozyma
			Schizoblastosporion
			Sympodiomyces
			Trigonopsis

[a] Authority names for genera are given in the individual chapters.

[b] A question mark preceding the genus name indicates that family assignment is uncertain.

[c] Placement in the class Hemiascomycetes is uncertain.

[d] The genus *Endomyces* and the family Endomycetaceae are uncertain, see discussion, this chapter.

ex Kurtzman is accepted over Schizosaccharomycetales O.E. Eriksson et al.

With the preceding changes, the class Hemiascomycetes has become phylogenetically circumscribed to include just the budding yeasts and such yeastlike genera as *Ascoidea* and *Cephaloascus*. Previously, all of these taxa were placed in the order Endomycetales. However, the identity of the Endomycetales has been in doubt because typification of *Endomyces*, the type genus, is based only on the drawings of Brefeld (von Arx 1972), rather than on a preserved specimen of collected type material. In an effort to preserve nomenclatural stability, Redhead and Malloch (1977) designated as neotype a specimen found on *Agaricus melleus* that appeared to match the description of *Endomyces decipiens*, the type species. Kurtzman and Robnett (1995) determined the 5′ end large subunit rDNA sequences from living cultures that had been identified as *Endomyces decipiens* and *Endomyces scopularum*. The strain identified as *E. decipiens* proved to be a member of the *Dipodascus albidus* clade, whereas *E. scopularum* was shown to be a euascomycete. Sequence analysis of the dried neotype of *E. decipiens*, although technically possible, will not resolve the taxonomic issue because of the uncertainty of which species is depicted in Brefeld's drawing. Because it is unknown whether *E. decipiens* is a member of the yeast clade or a euascomycete, or whether either of the living cultures that were sequenced were correctly identified, the order accepted for the budding and related yeasts is the Saccharomycetales as typified by the genus *Saccharomyces*.

Family assignments for members of the Saccharomycetales are generally uncertain because there are insufficient molecular data available to resolve most intergeneric relationships. Present assignments have been made using a combination of molecular and morphological similarities, but these assignments will change as more molecular data become available. To denote the uncertainty, many of the genera listed in Table 15 are preceded by a question mark. It should also be emphasized that at present there are insufficient molecular phylogenetic analyses to adequately circumscribe many of the genera. Consequently, most genus descriptions should be considered only as diagnostic groupings to aid in species identification. For example, the phylogenetically divergent genus *Pichia* is accepted with present species assignments only because proposals for its division into phylogenetically defined groups (genera) appear inadequate. Once additional information is available, some of the recently proposed genera, which are presently treated as synonyms, may be adopted. In another example, rDNA sequence comparisons suggest *Blastobotrys, Arxula* and *Sympodiomyces* to be congeneric and to represent the anamorphic states of *Stephanoascus* (Kurtzman and Robnett 1995). It should also be noted that rDNA-sequence comparisons have shown the genus *Oosporidium* to be a dimorphic euascomycete (Kurtzman and Robnett 1995). A history of each genus is given in individual chapters.

2. Diagnostic descriptions of presently accepted ascomycetous genera

2.1. Teleomorphic genera

2.1.1. *Ambrosiozyma*:

Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae with blastoconidia. True hypha have plugged septal pores that are usually visible under the light microscope as small dots.

Sexual reproduction: Asci are generally attached to hyphae, often in clusters, and they usually become deliquescent at maturity. Asci form 1–4 hat-shaped ascospores.

Physiology: Sugars are fermented, often slowly or weakly. Nitrate is assimilated by some species. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production and gelatin liquefaction are not determined.

2.1.2. *Arxiozyma*:

Vegetative reproduction: Multilateral budding; pseudohyphae may form but true hyphae are absent.

Sexual reproduction: Asci are unconjugated, persistent, and form 1–2 roughened, spheroidal ascospores.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin liquefaction is variable.

2.1.3. *Ascoidea*:

Vegetative reproduction: Budding cells and pseudohyphae are occasionally present; true hyphae are abundant and often form blastoconidia which may be sessile or borne on denticles.

Sexual reproduction: Asci are formed laterally or terminally on true hyphae and are ellipsoidal or acicular. Characteristically for this genus, new asci form percurrently inside older asci. Ascospores are hat-shaped or ellipsoidal, 16–160 per ascus, and are released through a terminal opening in the ascus.

Physiology: Sugars are not fermented. Nitrate may be assimilated. Pellicle formation on liquid media is not determined. Extracellular starch-like compounds are not produced. Inositol may be assimilated. Urease may be weakly produced. Gelatin liquefaction is not determined. The DBB reaction is ordinarily negative, but occasionally weak for *A. africana*.

2.1.4. *Babjevia*:

Vegetative reproduction: Multilateral budding; pseudohyphae are rudimentary; true hyphae are infrequently formed.

Sexual reproduction: Buds develop into asci while remaining attached to the parent cell. Several asci are usually

attached to a single parent cell, and the asci produce 4–30 hyaline, globose to ellipsoidal ascospores.

Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are sometimes produced. Inositol is not assimilated. Urease production and gelatin liquefaction are not determined. Cultures are not mucoid as is common for *Lipomyces*.

2.1.5. *Cephaloascus*:

Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae, often with blastoconidia which may be borne on distinct denticles.

Sexual reproduction: Erect ascophores are formed which may be smooth or roughened and hyaline or with a brownish pigment. Clusters of asci form at the apex of each ascophore to give a brush-like appearance. The asci, which become deliquescent, generally form 4 hat-shaped ascospores.

Physiology: Sugars may be fermented. Nitrate is not assimilated. Pellicles are formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.6. *Citeromyces*:

Vegetative reproduction: Multilateral budding; neither pseudohyphae nor true hyphae are formed.

Sexual reproduction: Asci are persistent and contain 1 or, infrequently, 2 roughened, spheroidal ascospores.

Physiology: Sugars are fermented. Nitrate is assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.7. *Clavispora*:

Vegetative reproduction: Multilateral budding; pseudohyphae may be formed, but true hyphae are not.

Sexual reproduction: Asci form following pairing of complementary mating types. The asci, which become deliquescent, produce 1–4 clavate ascospores.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.8. *Coccidiascus*:

Vegetative reproduction: Cells are spheroidal to ovoidal; pseudohyphae and true hyphae are not formed.

Sexual reproduction: Asci are thick-walled and contain 2 elongated ascospores that are closely intertwined to form a helix.

Physiology: Unknown. The taxon is known only from the tissue of *Drosophila*.

2.1.9. *Cyniclomyces*:

Vegetative reproduction: Cells are long-ovoidal to cylindrical with budding at the poles or on broad shoulders at the poles. Poorly differentiated pseudohyphae are present, but true hyphae are not formed.

Sexual reproduction: Asci are persistent and form 1–4 ovoidal to cylindrical ascospores.

Physiology: Sugars are weakly fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified. Growth occurs only between 30–40°C. Amino acids, B-vitamins, and an increased level of atmospheric CO_2 are required for growth.

2.1.10. *Debaryomyces*:

Vegetative reproduction: Multilateral budding; pseudohyphae may be formed but true hyphae are absent.

Sexual reproduction: Conjugation between a cell and its bud usually precedes ascus formation, but conjugation between independent cells may occur. Asci produce 1–4 ascospores that may be spheroidal or ellipsoidal and smooth or roughened, spheroidal and roughened with a prominent equatorial ring, or smooth and lenticular. Asci are persistent or deliquescent.

Physiology: Sugars are fermented by some species. Nitrate is not assimilated. Pellicles may be formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production and gelatin liquefaction are not determined.

2.1.11. *Dekkera* (Anamorph *Brettanomyces*):

Vegetative reproduction: Multilateral budding; cells are spheroidal to ellipsoidal, and some have an ogival shape. Pseudohyphae may form, but true hyphae are not produced. Single cells may profusely elongate and branch to give the appearance of a non-septate mycelium.

Sexual reproduction: Asci, which are unconjugated and become deliquescent, produce 1–4 ascospores that are hat-shaped or somewhat spheroidal with tangential brims.

Physiology: Sugars are fermented, and fermentation is usually stimulated by oxygen. Nitrate is assimilated by some strains. Pellicles may be formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is not produced. Gelatin liquefaction is not determined. Acetic acid is often produced. Growth is slow and generally short-lived.

2.1.12. *Dipodascopsis*:

Vegetative reproduction: Budding cells may be present. Pseudohyphae are absent, but true hyphae are abundant.

Sexual reproduction: Asci are acicular or cylindrical and form laterally on hyphae after fusion of gametangia. Ascospores are hyaline, ellipsoidal to reniform, and 32–128 are formed in each ascus. The spores are released following dissolution of the ascus apex.

Physiology: Sugars are not fermented. Nitrate is not

assimilated. Pellicle formation on liquid media is not determined. Extracellular starch-like compounds are produced. Inositol is assimilated. Urease production and gelatin liquefaction are not determined.

2.1.13. *Dipodascus* (Anamorph *Geotrichum*):

Vegetative reproduction: Arthroconidia and true hyphae are formed.

Sexual reproduction: Asci are acicular, cylindrical, ellipsoidal or subglobose and form after fusion of hyphal gametangia. Ascospores are hyaline and ellipsoidal; 4–128 are formed in each ascus. The spores are released following dissolution of the ascus apex.

Physiology: Fermentation of sugars is weak or absent. Nitrate is not assimilated. Pellicle formation on liquid media is not determined. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production and gelatin liquefaction are not determined.

2.1.14. *Endomyces*:

Vegetative reproduction: Pseudohyphae and true hyphae are present and may produce blastoconidia.

Sexual reproduction: Asci are subglobose to clavate and may arise following conjugation of hyphal gamatangia. Asci form 4–6 hat-shaped ascospores and deliquesce at maturity.

Physiology: Unknown. All known species appear to be obligate parasites of mushrooms (Agaricales).

2.1.15. Endomycete-like genera: See individual descriptions in chapter 31.

2.1.16. *Eremothecium*:

Vegetative reproduction: Multilateral budding and pseudohyphae are absent or present. True hyphae are formed.

Sexual reproduction: Asci are unconjugated, fusiform to cylindrical, become deliquescent, and produce 8–32 acicular to fusiform ascospores. Ascospores may have a central septum, and those of some species have a tapered, terminal extension of the cell wall.

Physiology: Sugars are fermented by some species. Nitrate is not assimilated. Pellicles may be formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is liquified by some species. Visible amounts of riboflavin are produced by some species.

2.1.17. *Galactomyces* (Anamorph *Geotrichum*):

Vegetative reproduction: Arthroconidia and true hyphae are produced.

Sexual reproduction: Asci are subspheroidal and arise following conjugation of hyphal gametangia. Asci produce 1 ascospore that is broadly ellipsoidal, sometimes with a faintly roughened surface, and a pale golden-brown color. Asci deliquesce at maturity.

Physiology: Sugars may be weakly fermented. Nitrate is not assimilated. Pellicle formation on liquid media is not determined. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production and gelatin liquefaction are not determined.

2.1.18. *Hanseniaspora* (Anamorph *Kloeckera*):

Vegetative reproduction: Bipolar budding; pseudohyphae may form but true hyphae are absent.

Sexual reproduction: Asci are unconjugated and form 1–4 ascospores that may be hat-shaped, or spheroidal and smooth, or rough and with or without an equatorial ledge. Asci may be persistent or deliquescent.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is not produced. Gelatin liquefaction is not determined.

2.1.19. *Issatchenkia*:

Vegetative reproduction: Multilateral budding; pseudohyphae are present, but true hyphae are not formed.

Sexual reproduction: Asci are unconjugated unless directly arising from the pairing of complementary mating types. Asci are persistent and form 1–4 spheroidal ascospores that may appear roughened under the light microscope.

Physiology: Glucose is fermented. Nitrate is not assimilated. Pellicles form early on liquid growth media. Extracellular starch-like compounds are not formed. Inositol is not assimilated. Urease production is not determined. Gelatin may be liquified.

2.1.20. *Kluyveromyces*:

Vegetative reproduction: Multilateral budding; pseudohyphae may be present, but true hyphae are not produced.

Sexual reproduction: Asci may or may not be conjugated and produce 1–4, or exceptionally, much greater than 4 ascospores that are smooth, reniform, bacilliform, ellipsoidal or spheroidal. Ascospores are liberated at maturity.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles may form on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.21. *Lipomyces*:

Vegetative reproduction: Multilateral budding; pseudohyphae are absent or rudimentary; true hyphae are absent.

Sexual reproduction: Buds, and occasionally the parent cells, develop into asci which produce 4–20 smooth or roughened ellipsoidal ascospores that are amber or brown in color. Asci generally deliquesce.

Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles may be formed on liquid media. Extracellular starch-like compounds are produced. Inositol may be assimilated. Urease production and gelatin liquifaction are not determined. Cultures are often quite mucoid.

2.1.22. *Lodderomyces*:

Vegetative reproduction: Multilateral budding; pseudohyphae are present but true hyphae are not produced.

Sexual reproduction: Asci are unconjugated, persistent and produce 1–2 long ellipsoidal ascospores.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.23. *Metschnikowia*:

Vegetative reproduction: Multilateral budding; pseudohyphae are usually present but true hyphae are not produced.

Sexual reproduction: Asci are unconjugated, persistent, and elongate with one end usually enlarged. Asci produce 1–2 needle-shaped ascospores that have no terminal appendages.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are formed infrequently on liquid media. Extracellular starch-like compounds are not formed. Inositol is not assimilated. Urease is not produced. Gelatin is not liquified.

2.1.24. *Nadsonia*:

Vegetative reproduction: Bipolar budding; pseudohyphae and true hyphae are not formed.

Sexual reproduction: Asci develop following conjugation between a cell and its bud. Either the bud, an opposing bud, or the cell becomes the ascus, which is persistent and forms 1–2 spheroidal ascospores that may be visibly roughened.

Physiology: Sugars may or may not be fermented. Nitrate is not assimilated. Pellicles are formed on liquid media. Extracellular starch-like compounds are not formed. Inositol is not assimilated. Urease is not produced. Gelatin is not liquified.

2.1.25. *Pachysolen*:

Vegetative reproduction: Multilateral budding; pseudohyphae may be poorly developed or absent; true hyphae are not produced.

Sexual reproduction: An ascus develops on the end of a refractile tube which arises from a vegetative cell that may or may not be conjugated. Asci produce 4 hat-shaped ascospores that are released through deliquescence of the ascus wall.

Physiology: Glucose and D-xylose are fermented. Nitrate is assimilated. Pellicles may form on liquid media. Extracellular starch-like compounds are not formed. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.26. *Pichia*:

Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae may be present.

Sexual reproduction: Asci may be conjugated or unconjugated, and persistent or deliquescent; 1–4, rarely 8, ascospores are produced that may be hat-shaped, hemispheroidal, or spheroidal and with or without a subequatorial ledge.

Physiology: Sugars are fermented by some species. Nitrate is assimilated by some species. Pellicles may form on liquid media. Extracellular starch-like compounds are not formed. Inositol is assimilated by one species. Urease production is not determined. Gelatin may be liquified.

2.1.27. *Protomyces*:

Vegetative reproduction: Budding is multilateral, but predominantly at the polar regions of cells. True hyphae are formed within the tissue of parasitized plants.

Sexual reproduction: Asci are formed within plant host tissue and produce up to several hundred ellipsoidal ascospores that appear to be forcibly discharged.

Physiology: Sugars are not fermented. Nitrate is assimilated by some species. Pellicles may form on liquid media. Extracellular starch-like compounds are generally formed. Inositol is not assimilated as a carbon source. Urease production is not determined. Gelatin may be weakly liquified.

2.1.28. *Saccharomyces*:

Vegetative reproduction: Multilateral budding; pseudohyphae may be present, but not true hyphae.

Sexual reproduction: Asci are persistent, generally unconjugated, and form 1–4 smooth, ellipsoidal ascospores.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not formed. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.29. *Saccharomycodes*:

Vegetative reproduction: Bipolar budding; pseudohyphae are absent or poorly developed; true hyphae are not formed.

Sexual reproduction: Asci are unconjugated, persistent, and produce 1–4 smooth, spheroidal ascospores with a minute subequatorial ledge.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not formed. Inositol is not assimilated. Urease is not produced. Gelatin may be weakly liquified.

2.1.30. *Saccharomycopsis*:

Vegetative reproduction: Multilateral budding and arthroconidia; pseudohyphae are present; true hyphae are abundant and often produce blastoconidia.

Sexual reproduction: Asci are usually unconjugated, generally deliquescent, and often attached to hyphae, or sometimes free and spindle-like. The asci produce 1–4, rarely 8, ascospores that may be hat-shaped, reniform with terminal appendages, spheroidal or ellipsoidal, and may have one or more ledges. Surfaces may be smooth or roughened.

Physiology: Sugars may or may not be fermented. Nitrate is not assimilated. Pellicles may be formed on liquid media. Extracellular starch-like compounds are not formed. Inositol may be assimilated. Urease production is not determined. Gelatin is not strongly liquified.

2.1.31. *Saturnispora*:

Vegetative reproduction: Multilateral budding; pseudohyphae may be produced but true hyphae are not.

Sexual reproduction: Asci are persistent or deliquescent and may be unconjugated or show conjugation between a cell and its bud or between independent cells. Asci produce 1–4, rarely 8, smooth, spheroidal ascospores that have an equatorial ledge.

Physiology: Glucose is fermented. Nitrate is not assimilated. Pellicles may be formed on liquid media. Extracellular starch-like compounds are not formed. Inositol is not assimilated. Urease production is not determined. Gelatin may be weakly liquified. D-xylose is not assimilated.

2.1.32. *Schizosaccharomyces*:

Vegetative reproduction: Cells divide by fission. True hyphae may develop and often fragment into arthrospores.

Sexual reproduction: Asci are usually unconjugated, deliquescent, and produce 2–8 spheroidal, ellipsoidal or reniform ascospores.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are produced. Inositol is not assimilated. Urease production and gelatin liquefaction are not determined.

2.1.33. *Sporopachydermia*:

Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae are not formed.

Sexual reproduction: Asci are deliquescent and may or may not be conjugated. The asci produce 1–4 spheroidal, subglobose, ellipsoidal or bacilliform ascospores.

Physiology: Glucose fermentation is absent or weak. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is assimilated as a sole carbon source. Urease production is not determined. Gelatin is not liquified.

2.1.34. *Stephanoascus*:

Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae are produced. Blastoconidia may form on the true hyphae.

Sexual reproduction: Asci are globose with an apical cap cell, persistent, and develop following fusion of hyphal gametangia. Ascospores are oblate to hat-shaped, and 2–4 are formed in an ascus.

Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicle formation on liquid media is not determined. Extracellular starch-like compounds are not produced. Inositol is assimilated by some species. Urease is not produced. Gelatin liquifaction is not determined.

2.1.35. *Torulaspora*:

Vegetative reproduction: Multilateral budding; pseudohyphae may be formed but true hyphae are not.

Sexual reproduction: Asci are persistent and may be unconjugated or show conjugation between a cell and its bud or between independent cells. Cells with tapered protuberances resembling conjugation tubes may also be present. Asci contain 1–4 spheroidal ascospores that are either roughened or smooth.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.36. *Wickerhamia*:

Vegetative reproduction: Bipolar budding; pseudohyphae and true hyphae are not formed.

Sexual reproduction: Asci are unconjugated, deliquescent, and form 1–2, rarely 16, ascospores that somewhat resemble a baseball cap in shape.

Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is not produced. Gelatin is not liquified.

2.1.37. *Wickerhamiella*:

Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae are not formed.

Sexual reproduction: Asci are conjugated and form 1 roughened, asymmetrical, elongated ascospore. Asci deliquesce at the terminus and appear corrugated following spore release.

Physiology: Sugars are not fermented. Nitrate is assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.38. *Williopsis*:

Vegetative reproduction: Multilateral budding; pseudohyphae may form but true hyphae are absent.

Sexual reproduction: Asci may be persistent or deliquescent and are unconjugated, or show conjugation between a cell and its bud or between independent cells. Asci produce 2–4 smooth, spheroidal or ellipsoidal ascospores that have an equatorial ledge.

Physiology: Glucose is fermented. Nitrate may or may not be assimilated. Pellicles are often formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin may be liquified. D-xylose is assimilated.

2.1.39. *Yarrowia*:
Vegetative reproduction: Multilateral budding; occasional formation of arthroconidia. Pseudohyphae and true hyphae are produced.
Sexual reproduction: Asci are unconjugated and slowly deliquesce at maturity. Asci form 1–4 ascospores that may be spheroidal, hemispheroidal, hat-shaped, or somewhat angular.
Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles are formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is strongly produced. Gelatin is strongly liquified. Lipases are produced.

2.1.40. *Zygoascus*:
Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae are formed.
Sexual reproduction: Asci are globose, persistent, and arise following conjugation between hyphal cells. Asci produce 1–4 ascospores that are hemispheroidal to hat-shaped.
Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles may be formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is assimilated. Urease is not produced. Gelatin liquefaction is not determined.

2.1.41. *Zygosaccharomyces*:
Vegetative reproduction: Multilateral budding; pseudohyphae may be formed but true hyphae are not.
Sexual reproduction: Asci are persistent and may be unconjugated or show conjugation between a cell and its bud; asci predominantly form by conjugation between independent cells, often with both cells producing ascospores. Ascospores are smooth, spheroidal to ellipsoidal, with 1–4 per ascus.
Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production is not determined. Gelatin is not liquified.

2.1.42. *Zygozyma*:
Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae are not formed.
Sexual reproduction: Asci, which become deliquescent, arise from evaginations on single cells or from the bridge of conjugating cells, and produce 4 to numerous smooth, elongate ascospores that show an amber color.
Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are produced. Inositol may be assimilated. Urease production and gelatin liquefaction are not determined.

2.2. Anamorphic genera

2.2.1. *Aciculoconidium*:
Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae with blastoconidia are present. Diagnostic of the genus is the presence of blastoconidia, each with a needle-shaped terminus and a rounded base.
Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicle formation on liquid media is not determined. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is not produced. Gelatin liquifaction is not determined.

2.2.2. *Arxula*:
Vegetative reproduction: Budding cells as well as arthroconidia are formed. Pseudohyphae and true hyphae are produced, the latter giving rise to blastoconidia that sometimes form on short denticles.
Physiology: Sugars are not fermented. Nitrate is assimilated. Pellicles may be formed on liquid media. Formation of extracellular starch-like compounds is not determined. Inositol is assimilated. Urease production and gelatin liquefaction are not determined.

2.2.3. *Blastobotrys*:
Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae are abundant. Diagnostic of the genus is production of blastoconidia on pedicels or denticles.
Physiology: Sugars are fermented by some species. Nitrate is not assimilated. Pellicle formation on liquid media is not determined. Inositol is assimilated by some species. Formation of extracellular starch-like compounds, urease production and gelatin liquefaction are not determined.

2.2.4. *Botryozyma*:
Vegetative reproduction: Multilateral budding; pseudohyphae are produced but true hyphae are absent. Diagnostic for this genus is the presence of terminal pseudohyphal cells that are dichotomously branched.
Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease production and gelatin liquefaction are not determined.

2.2.5. *Brettanomyces* (Teleomorph *Dekkera*):
Vegetative reproduction: Multilateral budding; cells are spheroidal to ellipsoidal, and some have an ogival shape. Pseudohyphae may form, but true hyphae are not produced.
Physiology: Sugars are fermented, and fermentation is usually stimulated by oxygen. Nitrate is assimilated by some strains. Pellicles may be formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is not produced. Gelatin liquefaction is not determined. Acetic acid is often produced. Growth is slow and generally short-lived.

2.2.6. *Candida*:
Vegetative reproduction: Multilateral budding; cells are globose, ellipsoidal or cylindroidal, and occasionally

ogival, triangular or lunate. Pseudohyphae and true hyphae may be formed. Arthroconidia are not formed.
Physiology: Sugars may be fermented. Nitrate may be assimilated. Pellicles may be formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is assimilated by some species. Urease is not produced. Gelatin may be liquified.

2.2.7. *Geotrichum* (Teleomorphs *Dipodascus, Galactomyces*):
Vegetative reproduction: Arthroconidia and true hyphae which disarticulate into arthroconidia.
Physiology: Sugars are fermented by some species. Nitrate is not assimilated. Pellicle formation on liquid media is not determined. Inositol is not assimilated. Formation of extracellular starch-like compounds, urease production, and gelatin liquefaction are not determined.

2.2.8. *Kloeckera* (Teleomorph *Hanseniaspora*):
Vegetative reproduction: Bipolar budding; pseudohyphae may form but true hyphae are absent.
Physiology: Sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is not produced. Gelatin liquefaction is not determined.

2.2.9. *Lalaria* (Teleomorph *Taphrina*):
Vegetative reproduction: Budding is on or near the poles of the cells. Growth is pinkish or sometimes yellowish in color.
Physiology: Sugars are not fermented. Inositol is not assimilated as a carbon source. Extracellular starch-like compounds are formed.

2.2.10. *Myxozyma*:
Vegetative reproduction: Multilateral budding; pseudohyphae are absent or rudimentary; true hyphae are not formed.
Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are produced. Inositol is assimilated by some species. Urease production is not determined. Gelatin may be liquified by some species. Cultures are often quite mucoid.

2.2.11. *Oosporidium*:
Vegetative reproduction: Multilateral budding; pseudohyphae and true hyphae may be present. Diagnostic of the genus is the presence of endospores, and growth that is light pink to light orange in color.
Physiology: Sugars are not fermented. Nitrate is assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are formed. Inositol is not assimilated. Urease production and gelatin liquefaction are not determined.

2.2.12. *Saitoella*:
Vegetative reproduction: Multilateral budding. Pseudohyphae and true hyphae are absent. Growth is orange to red in color.
Physiology: Sugars are not fermented. Nitrate is assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is produced. Gelatin liquefaction is not determined.

2.2.13. *Schizoblastosporion*:
Vegetative reproduction: Cell division is predominantly by bipolar budding. Pseudohyphae are absent or rudimentary; true hyphae are not formed.
Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is not produced. Gelatin liquefaction is not determined.

2.2.14. *Sympodiomyces*:
Vegetative reproduction: Budding is multilateral. Pseudohyphae and true hyphae are present. Diagnostic for the genus is the sympodial production of blastoconidia on short conidiophores that arise directly from yeast cells.
Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is assimilated. Urease is not produced. Gelatin is not liquified.

2.2.15. *Trigonopsis*:
Vegetative reproduction: Multilateral budding. Neither pseudohyphae nor true hyphae are produced. Diagnostic for the genus is the presence of triangular cells as well as ellipsoidal cells. Triangular cells bud at each apex.
Physiology: Sugars are not fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Extracellular starch-like compounds are not produced. Inositol is not assimilated. Urease is not produced. Gelatin liquefaction is not determined.

3. Key to the genera of ascomycetous yeasts

All species are DBB negative, except for the weak reaction shown by *Ascoidea africana*, and the positive reactions shown by *Sporopachydermia lactativora* and *S. quercuum* under some test conditions.

1. a Species are mycelial obligate parasites of mushrooms .... *Endomyces*: p. 194
   Endomycete-like genera: p. 197
   b Species are nonmycelial obligate parasites of *Drosophila* and characterized by linear ascospores coiled in a helix .... *Coccidiascus*: p. 153
   c Species are mycelial or nonmycelial, but can be grown on laboratory media → 2

2(1). a Cell division is exclusively by fission, i.e., cross wall formation without constriction → 3
   b Cell division is not exclusively by fission → 6

3(2). a Growth is butyrous and yeastlike . . . *Schizosaccharomyces*: p. 391
b Growth is predominantly mycelial; not exclusively yeastlike → 4
4(3). a Ascospores are not produced . . . *Geotrichum*: p. 574
b Ascospores are produced → 5
5(4). a Asci produce a single, ellipsoidal ascospore and completely deliquesce . . . *Galactomyces*: p. 209
b Asci produce 4–100-plus ellipsoidal ascospores and deliquesce at the tip . . . *Dipodascus*: p. 181
6(2). a Cell division is by bipolar budding on a broad base → 7
b Cell division is by multilateral budding, or not exclusively by bipolar budding on a broad base → 10
7(6). a Ascospores are formed → 8
b Ascospores are not formed → 9
8(7). a Ascospores are cap-shaped . . . *Wickerhamia*: p. 409
b Ascospores are smooth and spheroidal; asci are unconjugated, persistent, and form 4 spores, which often conjugate in pairs . . . *Saccharomycodes*: p. 372
c Ascospores are roughened and spheroidal; asci show conjugation between a cell and its bud, are persistent, and form 1–2 spores, often with a bud serving as an ascus . . . *Nadsonia*: p. 268
d Ascospores are hat-shaped, or roughened or smooth and spheroidal, and with or without an equatorial ledge; asci are unconjugated, persistent or deliquescent, and form 1–4 spores when hat-shaped or 2 when spheroidal . . *Hanseniaspora*: p. 214
9(7). a Glucose is fermented . . . *Kloeckera*: p. 580
b Glucose is not fermented . . . *Schizoblastosporion*: p. 602
10(6). a Budding cells are markedly triangular . . . *Trigonopsis*: p. 605
b Budding cells are not markedly triangular → 11
11(10). a Some budding cells have an ogival shape; cultures smell of acetic acid and are short-lived → 12
b Budding cells lack an ogival shape; cultures produce little or no acetic acid → 13
12(11). a Ascospores are formed . . . *Dekkera*: p. 174
b Ascospores are not formed . . . *Brettanomyces*: p. 450
13(11). a Ascospores are formed → 14
b Ascospores are not formed → 28
14(13). a Asci are borne on the tips of refractile tube-like cells . . . *Pachysolen*: p. 271
b Asci form brush-like clusters on the tips of erect ascophores . . . *Cephaloascus*: p. 143
c Asci form percurrently inside older asci . . . *Ascoidea*: p. 136
d Asci form between hyphae following conjugation, are globose, persistent and have an apical cap cell . *Stephanoascus*: p. 400
e Asci form between hyphae following conjugation, are globose, persistent, but lack an apical cap . . . *Zygoascus*: p. 422
f Asci are elongated, open by apical deliquescence, and produce 32–100-plus ellipsoidal to reniform spores *Dipodascopsis*: p. 178
g Asci not as above → 15
15(14). a Ascospores are needle-like, sometimes with a terminal extension; asci are deliquescent . . . *Eremothecium*: p. 201
b Ascospores are needle-like; asci are persistent . . . *Metschnikowia*: p. 256
c Ascospores are elongate, asymmetrical, roughened, and of minute size; asci deliquesce at the terminus and appear corrugated following release of the single spore . . . *Wickerhamiella*: p. 411
d Ascospores are clavate; asci are deliquescent . . . *Clavispora*: p. 148
e Ascospores not as above → 16
16(15). a Hyphal septa have plugged pores that appear as small dots under the light microscope . . . *Ambrosiozyma*: p. 129
b Hyphal septa, if present, do not have plugged pores → 17
17(16). a Asci are predominantly attached to hyphae, or if free, are spindle-shaped; gelatin is not strongly liquified . . . *Saccharomycopsis*: p. 374
b Asci may be free or attached to hyphae; strong liquefaction of gelatin . . . *Yarrowia*: p. 420
c Asci are usually free and not spindle-shaped; gelatin is not strongly liquified → 18
18(17). a Growth occurs between 30–40ºC; amino acids, B-vitamins, and an increased level of atmospheric CO_2 are required for growth . . . *Cyniclomyces*: p. 154
b Specialized growth conditions are not required → 19
19(18). a Asci arise as sac-like attachments on a cell → 20
b Asci are not as sac-like attachments → 21
20(19). a Cultures are usually mucoid; ascospores are amber; asci do not arise attached to the bridge of conjugating cells . . . *Lipomyces*: p. 248
b Cultures are usually mucoid; ascospores are amber; some asci arise attached to the bridge of conjugating cells *Zygozyma*: p. 433
c Cultures are not mucoid; ascospores are hyaline . . . *Babjevia*: p. 141
21(19). a Ascospores are spheroidal; asci are unconjugated and persistent → 22
b Ascospores are long ellipsoidal; asci are unconjugated and persistent . . . *Lodderomyces*: p. 254
c Ascospores are smooth, spheroidal or ellipsoidal, and have an equatorial ledge; asci may be conjugated and either deliquescent or persistent → 24
d Ascospores are hat-shaped or spheroidal with a small subequatorial ledge; asci are either deliquescent or persistent . . . *Pichia*: p. 273
e Ascospores and asci show combinations different from above → 25
22(21). a Early formation of pellicles on liquid media . . . *Issatchenkia*: p. 221
b No early formation of pellicles on liquid media → 23
23(22). a Ascospores are roughened; nitrate is assimilated . . . *Citeromyces*: p. 146
b Ascospores are roughened; nitrate is not assimilated . . . *Arxiozyma*: p. 134
c Ascospores are smooth; nitrate is not assimilated . . . *Saccharomyces*: p. 358

24(21). a D-Xylose is assimilated .... *Williopsis*: p. 413
b D-Xylose is not assimilated .... *Saturnispora*: p. 387
25(21). a Glucose fermentation is early and vigorous → 26
b Glucose fermentation is slow, weak, or absent → 27
26(25). a Asci are persistent and may be conjugated or unconjugated; ascospores are roughened or smooth and spheroidal; cells with tapered protuberances are often present .... *Torulaspora*: p. 404
b Asci are persistent, often conjugated, with conjugation predominantly between independent cells, both of which usually produce ascospores; ascospores are smooth and spheroidal to short ellipsoidal .... *Zygosaccharomyces*: p. 424
c Asci are deliquescent; ascospores are smooth and reniform, bacilliform, ellipsoidal or spheroidal ..... *Kluyveromyces*: p. 227
27(25). a Inositol is not assimilated as a sole source of carbon; ascospores are roughened or smooth, spheroidal or ellipsoidal, with or without an equatorial ledge, or smooth and lenticular; asci are usually conjugated and may be persistent or deliquescent .... *Debaryomyces*: p. 157
b Inositol is assimilated as a sole source of carbon; ascospores are smooth and spheroidal, subglobose, ellipsoidal, or bacilliform; asci are deliquescent .... *Sporopachydermia*: p. 395
28(13). a Cultures are red, pink, lavender or light yellow in color → 29
b Cultures are white or tannish-white in color → 30
29(28). a Cultures are salmon red; weak presence of extracellular starch-like compounds .... *Protomyces*: p. 353
b Cultures are salmon red; extracellular starch-like compounds are not present .... *Saitoella*: p. 600
c Cultures are pink, lavender or light yellow; extracellular starch-like compounds are present .... *Lalaria* (*Taphrina*): p. 582
d Cultures are light pink to light orange; cells with endospores are present .... *Oosporidium*: p. 598
30(28). a Cultures are mucoid; extracellular starch-like compounds are present .... *Myxozyma*: p. 592
b Cultures are not mucoid; extracellular starch-like compounds are not present → 31
31(30). a Blastoconidia with a needle-shaped terminus and a rounded base are present .... *Aciculoconidium*: p. 439
b Terminal pseudohyphal cells are dichotomously branched .... *Botryozyma*: p. 449
c Blastoconidia form on short denticles; nitrate is assimilated .... *Arxula*: p. 441
d Blastoconidia form on denticles; nitrate is not assimilated .... *Blastobotrys*: p. 443
e Sympodial production of blastoconidia on short conidiophores that arise directly from yeast cells; nitrate is not assimilated .... *Sympodiomyces*: p. 603
f Blastoconidia do not arise on markedly denticulate conidiophores and are not sympodially produced; nitrate may or may not be assimilated .... *Candida*: p. 454

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Chapter 16

A key to the anamorph genera of yeastlike Archi- and Euascomycetes

G.S. de Hoog

Contents

1. Introduction

A number of filamentous ascomycetous fungi (Euascomycetes) grow yeastlike in culture and may be confused with yeasts, which in the present book are conceived as either being of saccharomycetous or of basidiomycetous affinity. Euascomycetous anamorphs are distinguished from Saccharomycetes by having true hyphae with simple septal pores with Woronin bodies rather than micropores or dolipores, although in some genera of black yeasts Woronin bodies may be absent. Teleomorphs, where known, are perithecial or cleistothecial and belong to different orders of Ascomycetes. Hence the genera mentioned below are often of quite divergent relationships.

Ascospores are rarely produced in culture. The fungi mostly reproduce as Hyphomycetes by conidia, with additional budding cells. The main criteria for distinction of yeastlike Hyphomycetes are found in their morphology with the accent on details of conidiogenesis (Fig. 34). This classification does not correspond exactly with natural relationships based on teleomorph connections and supported by partial 18S rDNA sequence data. Some genera, thus, have to be taken as artificial entities (for example *Phialophora* and *Microdochium*), while in others the morphological groups have already been subdivided along natural lines (for example *Sporothrix*).

The following biological relationships have been established thus far:

(1) Archiascomycetes, order Taphrinales, genera *Taphrina* and *Protomyces* (Nishida and Sugiyama 1994, Nishida et al. 1995).

(2) Euascomycetes, order Dothideales, fam. Dothideaceae. The "black yeast" genera *Aureobasidium, Hormonema*, and probably *Hortaea* and the meristematic "black yeasts" *Botryomyces, Phaeotheca* and *Sarcinomyces* (de Hoog and Yurlova 1994, de Hoog et al. 1997b).

(3) Euascomycetes, order Chaeothyriales, fam. Herpotrichiellaceae. The "black yeast" genera *Exophiala, Phialophora, Rhinocladiella* and *Phaeococcomyces* (de Hoog 1977). Note that *Phaeococcomyces* is morphologically reminiscent of the basidiomycetous yeasts *Moniliella* and *Trichosporonoides*, which are distinguished by fermentation of glucose. *Phialophora* is phylogenetically heterogeneous, but a small group around the generic type species, *P. verrucosa*, contain herpotrichiellaceous species.

(4) Euascomycetes, order Ophiostomatales, fam. Ophiostomataceae. Genus *Sporothrix*. Note that basidiomycetous *Sporothrix*-like species are now classified in *Cerinosterus*, and saccharomycetous *Sporothrix*-like species in *Candida* (de Hoog 1992).

(5) Euascomycetes, Sphaeriales, fam. Coniochaetaceae. Most *Lecythophora* species probably are cultural states of members of this family (Mahoney and LaFavre 1981).

(6) Euascomycetes, Leotiales, fam. Leotiaceae. *Aureohyphozyma* is an anamorph genus of *Gelitanipulnivella* (Hosoya and Otani 1995). *Myriogonium* (Malloch and de Hoog, ch. 31, p. 198) and *Calyptrozyma* (Boekhout et al. 1995b) may also be related, the latter having some similarity to members of the Thelebolaceae.

Physiological patterns have been determined for a limited number of species. Diagnostic features are given in a simplified manner in the generic key below. Since characters may be variable within each genus, additional criteria are sometimes listed. For each genus, some references are given for further reading.

2. Key to yeastlike Archi- and Euascomycetes

1. a Conidiogenesis meristematic, by gradual swelling of the entire thallus, and with subsequent disarticulation; colonies slimy or moriform, restricted, black → 2
   b Conidiogenesis blastic or phialidic, by blowing out conidia; colonies more expanding, black, pink, yellow or white → 4

2(1). a Conidiogenesis endogenous; conidia liberated by disruption of parent cell walls; L-rhamnose and inulin not assimilated (Sigler et al. 1981) .... *Phaeotheca* Sigler, Tsuneda & Carmichael
   b Conidia in packets, liberated by disarticulation; L-rhamnose and inulin assimilated → 3

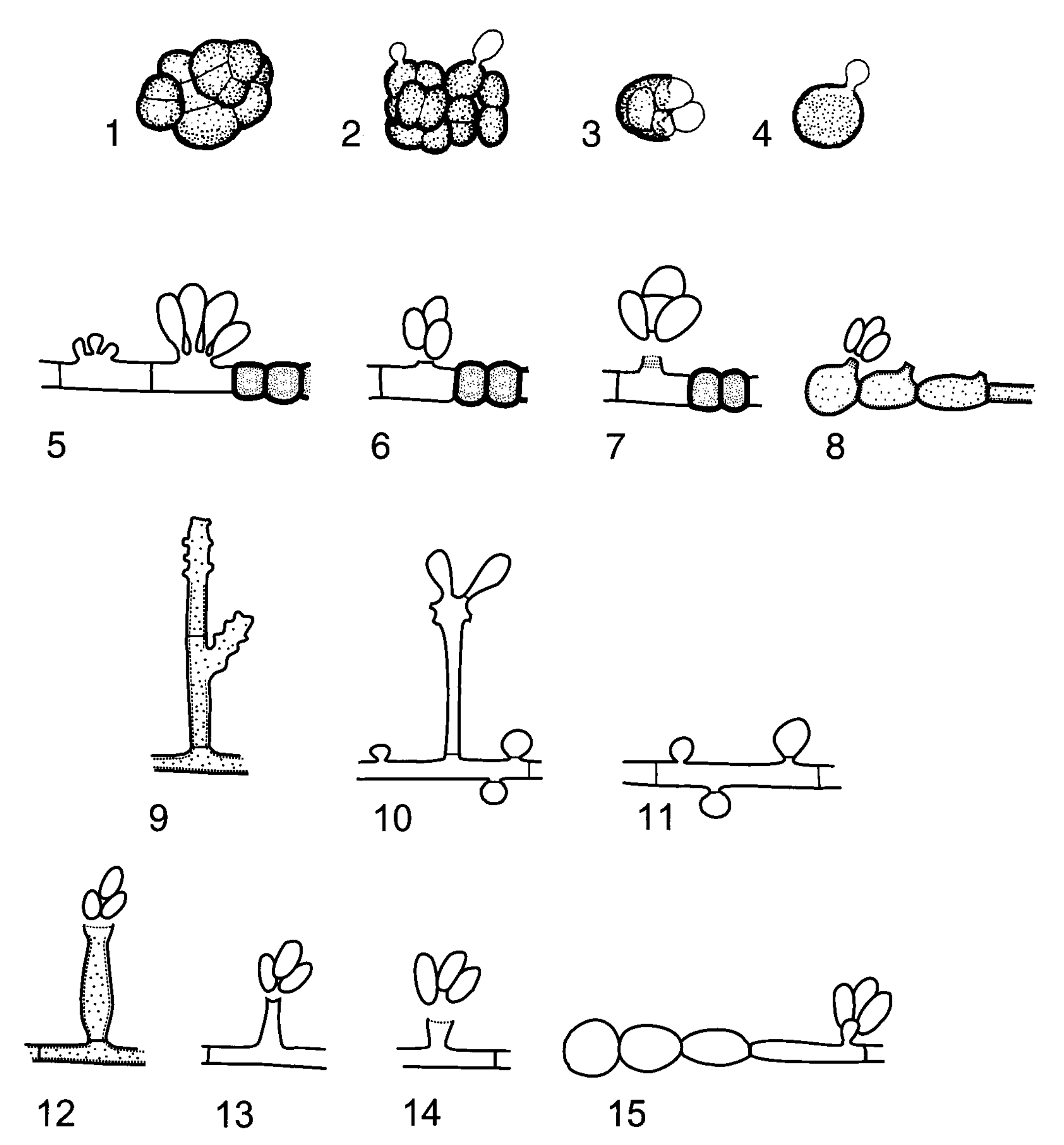


Fig. 34. Diagram of characteristic morphologies of anamorph genera of yeastlike Euascomycetes: (1) *Botryomyces*; (2) *Sarcinomyces*; (3) *Phaeotheca*; (4) *Phaeococcomyces*; (5) *Aureobasidium*; (6) *Hormonema*; (7) *Hortaea*; (8) *Exophiala*; (9) *Rhinocladiella*; (10) *Sporothrix*; (11) *Trichosporiella*; (12) *Phialophora*; (13) *Phialemonium*; (14) *Lecythophora*; (15) *Hyphozyma*.

3(2). a Conidial packets black, with septa at right angles; additional blastic conidia occasionally present; glucuronate assimilated (Sigler et al. 1981) .... *Sarcinomyces* Lindner

b Conidial packets initially whitish, cauliflower-like, with oblique septa; blastic conidia absent; glucuronate not assimilated (de Hoog and Rubio 1982, de Hoog and Guarro 1995) .... *Botryomyces* de Hoog & Rubio

4(1). a Hyphae absent or nearly absent → 5

b Hyphae predominant → 6

5(4). a Colonies pale pigmented, mostly pinkish or purplish (Kramer 1987) .... Taphrinales

b Colonies black (de Hoog 1977 (as *Phaeococcus*), de Hoog et al. 1995) .... *Phaeococcomyces* de Hoog

6(4). a Conidia sessile or on denticles, formed next to each other on the same cell (at least initially), occasionally in short chains that elongate at the apex → 7

b Conidia produced one after another through the same scar or opening and adhering in slimy balls (phialidic or annellidic) → 10

7(6). a Hyphae mostly over 3 μm wide, locally transformed into chlamydospores (Hermanides-Nijhof 1977, de Hoog and Yurlova 1994) .... *Aureobasidium* Viala & Boyer

b Hyphae mostly less than 3 μm wide; chlamydospores absent → 8

8(7). a Conidial apparatus dark olivaceous brown (de Hoog 1977, Veerkamp and Gams 1983) .... *Rhinocladiella* Nannfeld

b Conidial apparatus hyaline or subhyaline → 9

9(8). a Conidia close together on terminal denticles, often also alongside hyphae; erythritol and nitrate assimilated (de Hoog 1974, 1992) .... *Sporothrix* Hektoen & Perkins ex Nicot & Mariat

b Conidia scattered alongside hyphae only; erythritol and nitrate not assimilated (de Hoog et al. 1985) .... *Trichosporiella* Kamyschko ex W. Gams & Domsch

10(6). a Colonies olivaceous-black; hyphae regularly olivaceous → 11

b Colonies pale pinkish, reddish or yellowish, often later turning brown; hyphae hyaline or locally becoming pigmented → 13

11(10). a Expanding hyphae mostly less than 2.5 µm wide; conidia produced from inconspicuous annellated zones or from collarettes → 12

b Expanding hyphae often over 3 µm wide; annellated zones conspicuous, about 2 µm wide (Miyaji and Nishimura 1985, de Hoog and Gerrits van den Ende 1992) .... *Hortaea* Nishimura & Miyaji

12(11). a Conidia produced through pronounced collarette openings (Domsch et al. 1980, Wang and Zabel 1990, Yan et al. 1995) .... *Phialophora* Medlar

b Conidia produced from inconspicuous annellated zones (de Hoog 1977, de Hoog et al. 1995, de Hoog and Guarro 1995) .... *Exophiala* Carmichael

13(10). a Conidia produced from flat, barely visible openings → 14

b Conidia produced from hyaline collarettes (Gams and McGinnis 1983) .... *Lecythophora* Nannfeld

14(13). a Hyphae often over 3 µm wide, with local chlamydospores (Hermanides-Nijhof 1977) .... *Hormonema* Lagerberg & Melin

b Hyphae mostly less than 3 µm wide; chlamydospores absent → 15

15(14). a Colonies pink → 16

b Colonies whitish or cream-colored (Gams and McGinnis 1983) .... *Phialemonium* W. Gams & McGinnis

16(15). a Conidia formed on ampulliform cells; local clusters of brown chlamydospores present (Domsch et al. 1980) *Microdochium* H. Sydow

b Conidia formed alongside hyphae or on irregular protrusions; chlamydospores absent → 17

17(16). a Colonies pink, occasionally becoming brown (de Hoog and Smith 1981, 1986) .... *Hyphozyma* de Hoog & M.Th. Smith

b Colonies yellowish to olive-brown, then with yellow pigment exuded into the medium → 18

18(17). a Melibiose and ethylamine assimilated (Boekhout et al. 1995b) .... *Calyptrozyma* Boekhout & Spaaij

b Melibiose and ethylamine not assimilated (Hosoya and Otani 1995) .... *Aureohyphozyma* Hosoya & Otani

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Part Vb

Descriptions of teleomorphic ascomycetous genera and species

Contents

17. *Ambrosiozyma* van der Walt

M.Th. Smith

Diagnosis of the genus

Abundant formation of true mycelium with blastoconidia; in addition, budding yeast cells and pseudomycelium are present. Hyphal septa have a central, plugged pore surrounded by a thickening of the wall (dolipore-like). In light microscopy, the plugged pore shows as a small dark dot in the middle of the septum. Asci are generally formed on the hyphae. Ascospores are hat-shaped.

Sugars are fermented, often slowly and weakly. Nitrate is assimilated by some species. Diazonium blue B reaction is negative.

Type species

Ambrosiozyma monospora (Saito) van der Walt

Species accepted

1. *Ambrosiozyma ambrosiae* (van der Walt & D.B. Scott) van der Walt (1972)
2. *Ambrosiozyma cicatricosa* (D.B. Scott & van der Walt) van der Walt (1972)
3. *Ambrosiozyma monospora* (Saito) van der Walt (1972)
4. *Ambrosiozyma philentoma* van der Walt, D.B. Scott & van der Klift (1972)
5. *Ambrosiozyma platypodis* (J.M. Baker & Kreger-van Rij) van der Walt (1972)

Key to species

See Table 16.

1. a Nitrate assimilated . . . . . . *A. platypodis*: p. 132
   b Nitrate not assimilated → 2

2(1). a L-Rhamnose assimilated . . . . . . *A. philentoma*: p. 131
   b L-Rhamnose not assimilated → 3

3(2). a L-Arabinose assimilated → 4
   b L-Arabinose not assimilated . . . . . . *A. ambrosiae*: p. 129

4(3). a 2-Keto-D-gluconate assimilated . . . . . . *A. cicatricosa*: p. 130
   b 2-Keto-D-gluconate not assimilated . . . . . . *A. monospora*: p. 131

Table 16

Key characters of species assigned to the genus *Ambrosiozyma*

Species	Assimilation			
	L-Rhamnose	L-Arabinose	2-Keto-D-gluconate	Nitrate
Ambrosiozyma ambrosiae	–	–	–	–
A. cicatricosa	–	+	+	–
A. monospora	–	+	–	–
A. philentoma	+	–	–	–
A. platypodis	+	–	v	+

Systematic discussion of the species

17.1. *Ambrosiozyma ambrosiae* (van der Walt & D.B. Scott) van der Walt (1972)

Synonyms:

Pichia ambrosiae van der Walt & D.B. Scott (1971b)

Hormoascus ambrosiae (van der Walt & D.B. Scott) van der Walt & von Arx (1985)

Growth in malt extract: After 3 days at 25°C, the cells are spheroidal to ovoidal or short ellipsoidal (2.0–6.0)×(4.0–9.0–12.0) µm, and occur singly, in pairs or in small clusters. A sediment and occasionally a ring are formed. After one month at room temperature the culture consists of a thick sediment of yeast cells and mycelial hyphae.

Growth on malt agar: After one month at room temperature, the streak culture is butyrous, somewhat raised, rather dull, smooth or slightly rugose and gray-white to creamish-brown or occasionally pink in areas which sporulate heavily. The margin is slightly irregular without aerial mycelium.

Dalmau plate cultures on potato- and corn meal agar: Pseudohyphae as well as true, branched hyphae are produced in the agar under the coverslip. Clusters of small, spheroidal blastoconidia develop near or at the septa of the mycelial hyphae. Anastomoses are occasionally observed between the hyphae.

Formation of ascospores: Ascus formation is usually preceded by conjugation between independent cells. The zygote generally divides to form a short chain of cells, and one or sometimes several successive terminal cells serve as asci. The asci, which become deliquescent, usually produce four hat-shaped spores. Van der Walt and Scott (1971a) reported that single-spore isolates form sporogenous colonies, thus indicating the species to be homothallic.

Ascospores were observed on V8- and 5% Difco malt agars.

Fermentation:

Glucose	v	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

0.1% Cycloheximide	+	Growth at 35°C	v
Starch formation	–	Growth at 37°C	–

Co-Q: 7, CBS 6003 (Yamada et al. 1987b)
Mol% G+C: 34.7 (S.A. Meyer, cited in Barnett et al. 1990)

Origin of the strains studied: CBS 6003 (ATCC 24613; NRRL Y-7524), from tunnels of *Platypus externedentatus*, Zululand, South Africa, type strain of *Pichia ambrosiae;* CBS 6816, unknown, N.J.W. Kreger-van Rij; CBS 7108, from *Crossotarsus* sp. infestation of moribund *Ficus sycomorus,* South Africa, J.P. van der Walt; CBS 8256, from tunnels of *Crossotarsus externedentatus,* South Africa, J.P. van der Walt.

Type strain: CBS 6003 (ATCC 24613), isolated by van der Walt.

17.2. *Ambrosiozyma cicatricosa* (D.B. Scott & van der Walt) van der Walt (1972)

Synonym:
Pichia cicatricosa D.B. Scott & van der Walt (1971b)

Growth in malt extract: After 3 days at 25°C, cells are short-ovoidal to elongate, pear- and lemon-shaped, (3–8)×(6–14) µm, single or in pairs. Buds are formed on a broad base; more than one bud may be formed at the same site leading to multiple scars on the parent cell. Abundant branched mycelium with a diameter of 2.5–5 µm is present. A flocculant sediment is formed. After one month at 17°C a thick lumpy mass is present. The culture has a fragrant odor.

Growth on malt agar: After one month at 17°C, the streak is cream colored to yellow, tough, dull and raised in the middle. The edge is fringed with mycelium.

Dalmau plate cultures on potato- and corn meal agar: Branched mycelium is formed with groups of spheroidal to elongate blastoconidia at the end of the hyphae or alongside them, often in loose groups. Some blastoconidia produce a series of buds at the top and show polar elongation with multiple scars, the dactyloid cells (Scott and van der Walt 1971b). Pseudomycelium may be present.

Formation of ascospores: Asci are spheroidal or short-ovoidal and situated singly or in groups, occasionally among blastoconidia, at the end of the hyphae or alongside hyphae at the septa. Asci contain one to four, often two hat-shaped spores which are generally not liberated. Spores measure (5–6)×(10–12) µm, including the brim (Scott and van der Walt 1971b).

Ascospores were observed on corn meal, oatmeal, and V8 agars.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	v	Trehalose	v
Maltose	s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

0.1% Cycloheximide	+	Growth at 37°C	+
Starch formation	–		

Co-Q: Not determined.

Mol% G+C: 40.2 (S.A. Meyer, cited in Barnett et al. 1990)

Origin of the strains studied: CBS 6157 (ATCC 24611), from tunnels of *Xyleborus torquatus* in *Cussonia umbelifera*, Natal, South Africa, J.P. van der Walt, type strain of *Pichia cicatricosa*; CBS 6158, from tunnels of *Xyleborus torquatus* and *Platypus externedentatus* in *Macaranga capensis,* South Africa, received from J.P. van der Walt.

Type strain: CBS 6157.

17.3. *Ambrosiozyma monospora* (Saito) van der Walt (1972)

Synonyms:

Endomycopsis monospora Saito (1932)
Endomycopsis fibuligera (Lindner) Dekker var. *monospora* (Saito) Lodder & Kreger-van Rij (1952)
Pichia monospora (Saito) Pignal (1968)
Endomyces bispora Verrall (1943) [nec *Endomyces bisporus* Beck (1922)]
Endomycopsis fasciculata Batra (1963a)
Pichia fasciculata (Batra) Boidin, Abadie & Lehodey (1965a)
Pichia crossotarsi Batra (1971)

Growth in malt extract: After 3 days at 25°C, the culture consists of mycelial hyphae with side branches with a diameter of 1.5–5.5 μm, and spheroidal to short-ovoidal, budding yeast cells measuring (7–9)×(7–13) μm, single, in pairs or in chains. Pseudomycelium may also be present. The culture has a fragrant odor. A loose, flocky sediment is formed. After one month at 17°C a thick, lumpy sediment, filling up the liquid, and a broad ring are present.

Growth on malt agar: After one month at 17°C, the streak culture is yellow to yellow-brown, tough, raised, dull, and delicately wrinkled over the whole surface. The margin is fringed with mycelium.

Dalmau plate cultures on potato- and corn meal agar: True, branched mycelium is formed. Spheroidal to short-ovoidal blastoconidia occur in chains or in clusters at the end of the mycelial hyphae or alongside them. Small protuberances may occur on the hyphae. Pseudomycelium may be present.

Formation of ascospores: Asci are spheroidal or ovoidal, and are situated at the end of the mycelial hyphae or alongside them at the septa. Single spores give cultures which sporulate again (Batra 1963a). Spores are hat-shaped; one to two are formed per ascus. They measure, including the brim, (3–4)×(6–8) μm, and are released from the ascus. The presence of many spores give the culture a brownish appearance.

Ascospores were observed on 5% Difco malt-, corn-meal-, potato-, and YM agars.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	v	Trehalose	+
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

0.1% Cycloheximide	+	Growth at 37°C	+
Starch formation	–		

Co-Q: 7 (Yamada et al. 1976b)

Mol% G+C: 38.0 (T_m: Nakase and Komagata 1970b); 38.3–39.5 (S.A. Meyer, cited in Barnett et al. 1990)

Origin of the strains studied: CBS 2554 (ATCC 56618; NRRL Y-1484), exudate of coconut palm; CBS 5514 (ATTC 14628, IFO 1965, NRRL Y-5955), type strain of *Endomycopsis fasciculata,* from *Pinus strobus,* L.R. Batra; CBS 5515 (ATCC 56460, NRRL Y-1081), type strain of *Endomyces bispora,* tunnels of *Platypus compositus,* L.J. Wickerham; CBS 6392 (ATCC 18855), type strain of *Pichia crossotarsi,* ambrosia fungus of *Crossotarsus wollastoni.*

Type strain: CBS 2554, the original strain described as *Endomycopsis monospora* by Saito and isolated from coconut palm.

17.4. *Ambrosiozyma philentoma* van der Walt, D.B. Scott & van der Klift (van der Walt 1972)

Synonym:

Hormoascus philentomus (van der Walt, D.B. Scott & van der Klift) van der Walt & von Arx (1985)

Growth in malt extract: After 3 days at 25°C, cells are spheroidal, short-ovoidal and ovoidal, (3.5–7)×(5–10) μm, single, in pairs or small chains. Pseudomycelium and true mycelium, may be present. A loose sediment is present. After one month at 17°C a thick lumpy sediment filling up the liquid is formed. The culture has a fragrant odor.

Growth on malt agar: After one month at 17°C, the streak culture is cream colored to brownish, tough, raised, slightly wrinkled or smooth and dull. The margin is fringed with mycelium.

Dalmau plate cultures on potato- and corn meal agar: Abundant formation of branched mycelium with spheroidal to ovoidal and long-ovoidal blastoconidia at the ends of hyphae, along them, at the septa in between, or often in small branched chains. Pseudomycelium, may be present.

Formation of ascospores: Fusion between loose yeast cells occurs. Asci are spheroidal or ovoidal, situated terminally or laterally on the hyphae, often in small chains. They contain one to four, usually four, hat-shaped ascospores which are easily liberated. According to van der Walt (1972), the species is homothallic.

Ascospores were observed on V8-, 5% Difco malt-, and corn meal agars.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	v	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

0.1% Cycloheximide	+	Growth at 30°C	+
Starch formation	–	Growth at 35°C	–

Co-Q: 7 (Goto and Takami 1986)
Mol% G+C: 35.1 (S.A. Meyer, cited in Barnett et al. 1990)

Origin of the strains studied: CBS 6276 (ATCC 24607, IFO 1847, NRRL Y-7523); CBS 6279, tunnel of *Xyleborus aemulus,* South Africa, J.P. van der Walt.

Type strain: CBS 6276, received from J.P. van der Walt.

17.5. *Ambrosiozyma platypodis* (J.M. Baker & Kreger-van Rij) van der Walt (1972)

Synonyms:
Endomycopsis platypodis J.M. Baker & Kreger-van Rij (1964)
Hansenula platypodis (J.M. Baker & Kreger-van Rij) Fiol (1967)
Hormoascus platypodis (J.M. Baker & Kreger-van Rij) von Arx (1972)

Growth in malt extract: After 3 days at 25°C, the cells are spherical, 5–9 μm, single or in pairs. Mycelial hyphae and pseudomycelium are also present. A sediment and a ring are formed. The culture has a sour fruity odor.

Growth on malt agar: After one month at 17°C, the streak culture is yellow with an orange tinge, tough, raised in the middle, dull and strongly plaited; the edge is fringed with mycelium.

Dalmau plate cultures on potato- and corn meal agar: True branched mycelium with spheroidal, ovoidal to long-ovoidal, blastoconidia is abundantly formed. The blastoconidia occur singly, in clusters or in short chains near the septa or in between. Anastomoses between the hyphae may occur. Pseudomycelium may be present.

Formation of ascospores: Conjugation between yeast cells may occur. The zygote forms buds or hyphae; the former may turn directly into asci, the latter may bear spheroidal or ovoidal asci terminally or laterally, often in small clusters. Anastomoses between hyphae may also occur. Asci contain one to four, usually four, hat-shaped spores which are easily released from the ascus. The presence of many spores gives the culture a brownish color. Single ascospores give cultures which sporulate again.

Ascospores were observed on 5% Difco malt-, corn-meal-, potato and YM agars.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

0.1% Cycloheximide	+	Growth at 35°C	v
Starch formation	–	Growth at 37°C	–

Co-Q: 7 (Yamada et al. 1976b)
Mol% G+C: 28.5–33.4, 4 strains (T_m: Nakase and Komagata 1969); 36.6–36.8 (Nakase and Komagata 1971b); 38.3–39.5 (S.A. Meyer, cited in Barnett et al. 1990)

Origin of the strains studied: CBS 4111 (ATCC 24747; NRRL Y-6732), tunnel of *Platypus cylindrus,* UK, J.M. Baker; CBS 4380, ex CBS 4111, J. Lodder; CBS 7105, CBS 7106, from *Platypus* sp. in *Notophagus cunninghamii,* Tasmania, G.S. de Hoog.

Type strain: CBS 4111, isolated by J.M. Baker

Comments on the genus

Van der Walt (1972) introduced *Ambrosiozyma* to accommodate mycelial yeast species which are associated with bark beetles and are characterized by hyphal septa with dolipores. He transferred *Pichia ambrosiae* and *P. cicatricosa,* as well as some *Endomycopsis* species, such as *E. monospora* and *E. platypodis,* to this genus. Shortly thereafter, von Arx (1972) described *Hormoascus* to accommodate the nitrate assimilating *A. platypodis.* Kreger-van Rij (1984b) retained *A. platypodis* in the genus *Ambrosiozyma* but excluded *A. ambrosiae* because Kreger-van Rij and Veenhuis (1973) were unable to find dolipores. Consequently, Kurtzman (1984d) retained this latter species in *Pichia*. Van der Walt and von Arx (1985) emended *Hormoascus* to include nitrate-positive and nitrate-negative species and transferred *P. ambrosiae, A. platypodis* and *A. philentoma* to *Hormoascus.* Kurtzman and Robnett (1994a, 1995) demonstrated from comparisons of rRNA/rDNA sequences that *Ambrosiozyma* and *Hormoascus* are congeneric.

18. *Arxiozyma* van der Walt & Yarrow

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or occasionally elongate. True hyphae are not produced, but pseudohyphae may form. Pellicles are not formed on the surface of liquid media.

Asci are unconjugated, persistent, and produce one, or rarely two, spheroidal ascospores. Ascospores are verrucose to tuberculate.

Glucose is fermented. Coenzyme Q-6 is produced. Diazonium blue B reaction is negative.

Type species

Arxiozyma telluris (van der Walt) van der Walt & Yarrow

Species accepted

1. *Arxiozyma telluris* (van der Walt) van der Walt & Yarrow (1984)

Systematic discussion of the species

18.1. *Arxiozyma telluris* (van der Walt) van der Walt & Yarrow (1984b)

Anamorph: *Candida pintolopesii* (van Uden) S.A. Meyer & Yarrow
Synonyms:
Saccharomyces telluris (as *S. tellustris*) van der Walt (1957)
Torulopsis pintolopesii van Uden (1952)
Candida pintolopesii (van Uden) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Candida bovina van Uden & do Carmo-Sousa (1957)
Torulopsis bovina (van Uden & do Carmo-Sousa) van Uden & Vidal-Leiria (1970)
Candida slooffii van Uden & do Carmo-Sousa (1957)
Torulopsis pintolopesii van Uden var. *slooffii* Mendonça-Hagler & Phaff (1975) nom. inval.
Candida pintolopesii (van Uden) S.A. Meyer & Yarrow var. *slooffiae* (van Uden & do Carmo-Sousa) Mendonça-Hagler & Phaff ex S.A. Meyer, Ahearn & Yarrow (1984)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal, or infrequently elongate $(2.9–6.0)\times(4.5–7.5)$ µm, and single, in pairs, or in small clusters. Growth is butyrous, dull-glistening, and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows no true hyphae. Pseudohyphae may be formed by some strains. Aerobic growth is white to tannish-white, faintly glistening, and butyrous. Single colonies are low convex with a depressed center. A pleasant, faintly acidic odor is present.

Formation of ascospores: Asci are transformed from unconjugated vegetative cells and produce one, or infrequently two, spheroidal ascospores. Asci are persistent (Fig. 35). Transmission electron microscopy shows ascospore surfaces to be roughened by protuberances that arise from the outer layer of the spore wall (van der Walt and Yarrow 1984b). It is not known whether this species is homothallic or heterothallic.

Ascospores were observed on McClary's acetate, YM, and V8 agars after 4–7 days at 25°C. Acetate agar gave markedly better ascosporulation than the other media tested.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	w/–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 6, (Yamada et al. 1976b).
Mol% G+C: 32.3–34.0, 14 strains (BD: Mendonça-Hagler and Phaff 1975).

Origin of the strains studied: CBS 2685 (NRRL YB-4302), soil isolate, Cape Province, South Africa,

from J.P. van der Walt; NRRL Y-7282 (CBS 6264), NRRL Y-7283, NRRL Y-7284 (CBS 6258), NRRL Y-7285, all from the nasal cavities of pigeons, U.S.A., from H.F. Hasenclever; NRRL Y-11611 (CBS 2675), from rodent droppings, New Zealand; NRRL Y-11612 (CBS 2676), mouse mammary gland, Netherlands; NRRL Y-11613 (CBS 2985), peritoneal fluid of a dead guinea pig, Indonesia; NRRL Y-11614 (CBS 5812), from a man, France.

Type strain: CBS 2685, from soil, South Africa.

Comments on the genus

Arxiozyma telluris was initially isolated and described by van der Walt (1957) as *Saccharomyces tellustris*. The original spelling of the species name was treated as an orthographic error and changed to *telluris* (van der Walt 1970d). Kreger-van Rij (1966b) and Kurtzman et al. (1980b) reported the ascospore walls of *S. telluris* to have surface protuberances, and with the redescription of *Saccharomyces* by Yarrow (1984a), *S. telluris* became the only species in the genus to have roughened ascospore walls. Because of this unique ascospore topology, van der Walt and Yarrow (1984b) proposed that *S. telluris* was phylogenetically isolated and they described the new genus *Arxiozyma*.

Kreger-van Rij (1958) proposed that *S. telluris* represented the teleomorph of *Candida bovina*, noting the phenotypic similarity between the two species as well as ascosporulation in one strain of *C. bovina*. Mendonça-Hagler and Phaff (1975) compared the extent of nDNA complementarity between *S. telluris, C. bovina, C. slooffii* and *Torulopsis pintolopesii* and showed that all species shared 80% or greater nDNA relatedness. Although Mendonça-Hagler and Phaff (1975) considered all four taxa to be conspecific, they proposed designating *C. slooffii* as a variety of *T. pintolopesii* because strains of *C. slooffii* consistently differed from other strains of the species by having a minimum growth temperature of 28°C, by exhibiting an absolute requirement for inositol, and by formation of pseudomycelium.

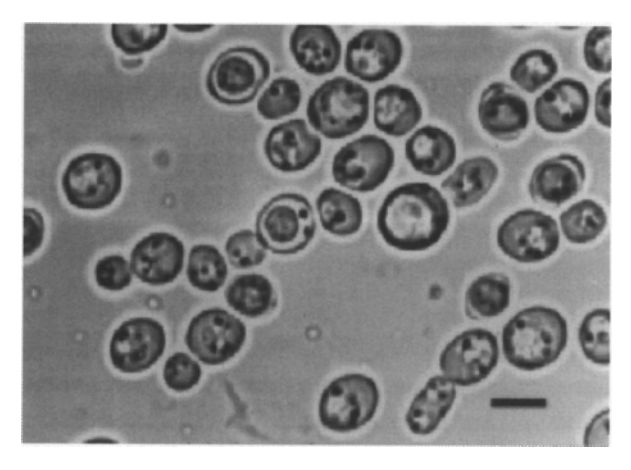


Fig. 35. *A. telluris*, CBS 2685. Unconjugated asci, each with a spheroidal ascospore. After 1 week on McClary's acetate agar at 25°C. Bar = 5 µm.

19. *Ascoidea* Brefeld & Lindau

G.S. de Hoog

Diagnosis of the genus

Colonies are smooth, moist or dry, mostly with an expanding, submerged mycelium. Species are often dimorphic, with colonies being restricted and yeastlike or expanding and hyphal. Budding cells and pseudomycelium are present or absent. Wide, true hyphae are present and form blastoconidia which are sessile or on denticles and occur singly or in short, branched chains.

Asci are lateral or terminal on hyphae, ellipsoidal or acicular, with firm walls, and contain numerous ascospores which are liberated through a terminal opening; new asci are formed percurrently inside the remains of a previous ascus. Ascospores are ellipsoidal, with a unilateral, mucilaginous brim.

Fermentation is absent. Urease is absent. Diazonium blue B reaction is negative, rarely weak.

Type species

Ascoidea rubescens Brefeld

Species accepted

1. *Ascoidea africana* Batra & Francke-Grosmann (1964)
2. *Ascoidea corymbosa* W. Gams & Grinbergs (1970)
3. *Ascoidea hylecoeti* Batra & Francke-Grosmann (1961)
4. *Ascoidea rubescens* Brefeld (1891)

Key to species

See Table 17.

Morphological key:

1. a Conidia 23–38 µm long .... *A. rubescens*: p. 139
 b Conidia less than 15 µm long → 2
2(1). a Asci obclavate .... *A. hylecoeti*: p. 138
 b Asci ellipsoidal to broadly clavate → 3
3(2). a Ascospores (2.5–4.0)×(3.5–5.5) µm .... *A. africana*: p. 137
 b Ascospores (2.3–2.6)×(2.8–3.6) µm .... *A. corymbosa*: p. 137

Physiological key:

1. a Sucrose assimilated → 2
 b Sucrose not assimilated → 3
2(1). a Lactose assimilated .... *A. rubescens*: p. 139
 b Lactose not assimilated .... *A. corymbosa*: p. 137
3(1). a Citrate assimilated .... *A. africana*: p. 137
 b Citrate not assimilated .... *A. hylecoeti*: p. 138

Table 17
Key characters to species in the genus *Ascoidea*

Species	Assimilation								Tolerance to 10% NaCl
	Sucrose	Maltose	Lactose	D-Arabinose	D-Ribose	Erythritol	Citrate	Nitrate	
Ascoidea africana	–	–	–	–	–	–	+	–	+
A. corymbosa	+	+	–	–	–	–	–	+	–
A. hylecoeti	–	–	–	–	–	–	–	–	–
A. rubescens	+	+	+	+	+	+	–	–	–

Systematic discussion of the species

19.1. *Ascoidea africana* Batra & Francke-Grosmann (1964)

Growth on 4% malt extract agar: After 10 days at 20–22°C, colonies are cream colored, smooth, thin, moist at the center and with a flat, somewhat farinose marginal zone. Hyphae are 3–5 µm wide, irregularly branched, and bear groups of blastoconidia at irregular distances. Conidia are sessile, clavate, of variable size, and bear a dense cluster of ellipsoidal secondary conidia on small denticles at the tip; secondary conidia are about 3×5 µm.

Formation of ascospores: Asci are formed singly or in small whorls alongside hyphae, mostly inserted just below distal septa, and broadly ellipsoidal, (8–13)×(20–30) µm, with firm walls containing 16–70 ascospores which are liberated by apical deterioration of the ascus. Ascospores are hat-shaped, (2.5–4.0)×(3.5–5.5) µm, cohering in slimy balls after liberation. New asci are formed percurrently 2 to 3 times inside the remains of a previous ascus, and the wall remnants become retracted (Fig. 36). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	w
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	w	Ribitol	+
Trehalose	+	Galactitol	n
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	w	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	w	Vitamin-free	n

Additional assimilation tests and other growth characteristics:

Arbutin	+	Nitrite	–
L-Arabinitol	+	Ethylamine	+
Glucono-δ-lactone	+	L-Lysine	+
D-Galacturonate	–	Cadaverine	+
Urease	w	Creatine	–
10% NaCl	+	Creatinine	–
10% $MgCl_2$	+	Starch formation	–
0.01% Cycloheximide	–	Growth at 30°C	–
DBB	w		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: CBS 377.68 (ATCC 24275), from larva, West Africa, Batra.

Type strain: CBS 377.68.

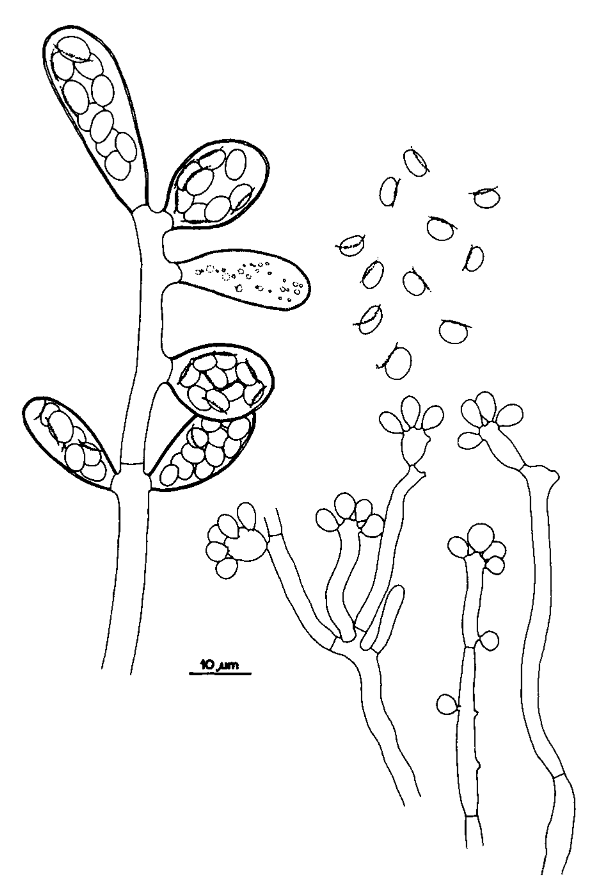


Fig. 36. *A. africana*, CBS 377.68. Asci, liberated ascospores and true hyphae with clusters of conidia. Malt extract agar, 22°C, 30 days.

Comments: The species grows poorly in liquid medium and physiological test results were therefore judged with difficulty. The type strain was isolated from a larva infesting *Chlorophora excelsa* wood from West Africa, imported into Germany. The species is probably an ambrosia fungus disseminated by insect vectors.

19.2. *Ascoidea corymbosa* W. Gams & Grinbergs (1970)

Growth on 4% malt extract agar: After 10 days at 20–22°C, colonies are cream colored, smooth, slimy, and with or without expanding submerged mycelium. Hyphae are 4–5 µm wide with lateral branches, 2.5–3.5 µm wide, bearing whorls of sessile blastoconidia in short, branched, acropetal chains just below the septa. Conidia are ellipsoidal, (2.5–3.5)×(4–8) µm. Budding is mostly absent.

Formation of ascospores: Asci are formed in small groups in distal portions of hyphae. They are mostly ellipsoidal, (10–14)×(20–40) µm, with firm walls and contain 16–40 ascospores which are liberated by apical deterioration of the ascus. Ascospores are hat-shaped, (3.0–3.5) µm in diameter, and cohere in dense slimy balls after liberation. New asci are formed percurrently 2 to 3 times inside the remains of a previous ascus; the wall

remnants are extended or retracted (Fig. 37). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	w
L-Sorbose	w	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	w	Galactitol	n
Lactose	–	D-Mannitol	–
Melibiose	v	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	w
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	v	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	w	Vitamin-free	n

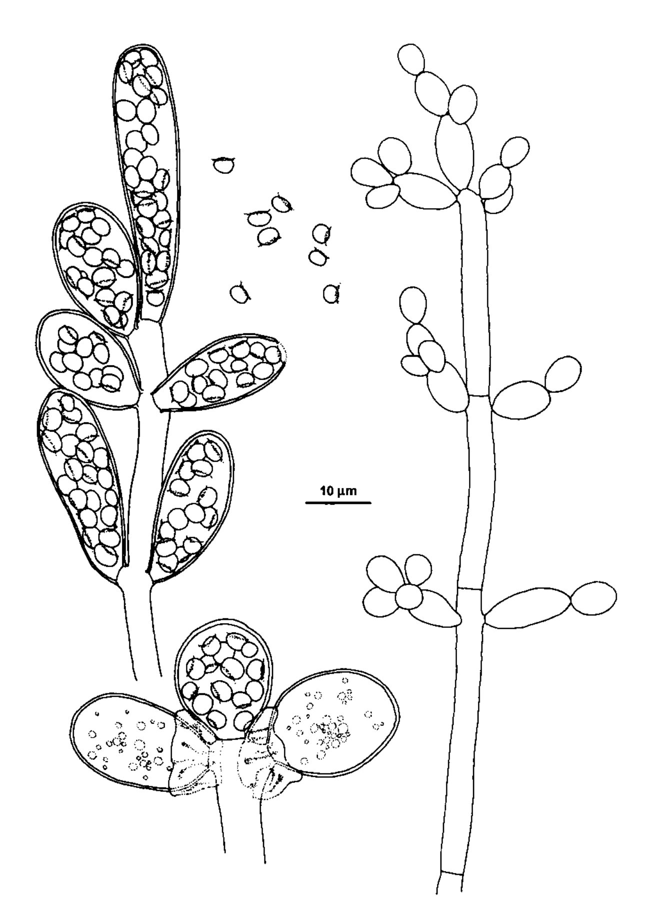


Fig. 37. *A. corymbosa*, CBS 177.70. Asci, in part deteriorating apically, with new asci arising through retracted remains of the previous ascus. Liberated ascospores and true hyphae with clusters of conidia. Malt extract agar, 22°C, 3 weeks.

Additional assimilation tests and other growth characteristics:

Arbutin	+	Nitrite	–
L-Arabinitol	v	Ethylamine	+
Glucono-δ-lactone	w	L-Lysine	+
D-Galacturonate	–	Cadaverine	+
Urease	–	Creatine	w
10% NaCl	–	Creatinine	w
10% $MgCl_2$	w	Starch formation	–
0.01% Cycloheximide	–	Growth at 30°C	–
DBB	–		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 457.69 (ATCC 24275) and three additional strains, all from decaying wood of *Araucaria araucana*, Chile, Grinbergs.

Type strain: CBS 457.69.

Comments: The species has only been isolated from rotten *Araucaria* wood in Chile. Cultures show a remarkable dimorphism. Some strains formed a large zone with submerged, expanding hyphae while others mainly consisted of more typical, restricted, slimy microcolonies. Hyphal strains show little growth in submersion and physiological test results were therefore not presented. The species was synonymized with *A. africana* by von Arx and Müller (1984) but differs by smaller ascospores and by physiological characters listed in Table 17.

19.3. *Ascoidea hylecoeti* Batra & Francke-Grosmann (1961)

Growth on 4% malt extract agar: After 10 days at 20–22°C, colonies are cream colored, restricted (4 mm diameter), and slimy or expanding (15 mm) and thinly floccose. Slimy colonies consist of budding cells and fragile pseudomycelium bearing conidia in groups on large, butt-shaped denticles. Dry colonies are comprised of sparingly septate hyphae which are up to 8 µm wide and often have irregular protrusions.

Formation of ascospores (Batra and Francke-Grosmann 1961)**:** Asci are borne terminally on erect hyphae later becoming lateral due to further growth of supporting hypha; asci are formed in percurrent succession and are obclavate, (15–24)×(160–400) µm, apex 3–4.5 µm wide, base 12–18 µm wide, and contain 150–400 ascospores. Asci open by terminal deterioration and liberate a cirrhus of ascospores (Fig. 38). Ascospores are ellipsoidal, (1.5–2.0)×(2.5–3.2) µm and appear hat-shaped due to a unilateral mucilaginous fringe.

Fermentation: absent.

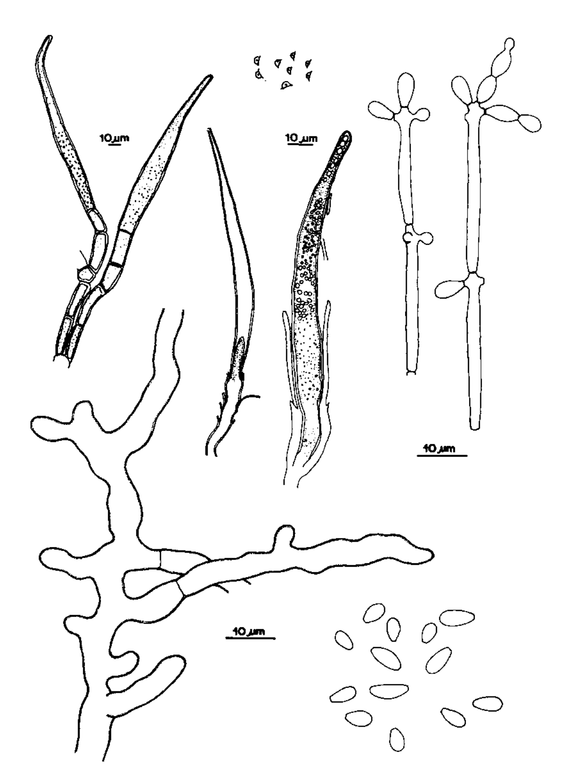


Fig. 38. *A. hylecoeti*, CBS 355.80. Asci, in part being produced through remains of a previous ascus; ascospores (from Batra and Francke-Grosmann 1961); true, non-sporulating hyphae, pseudomycelial budding state.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	−	Methanol	w
L-Sorbose	+	Ethanol	+
Sucrose	−	Glycerol	+
Maltose	−	Erythritol	−
Cellobiose	+	Ribitol	w
Trehalose	+	Galactitol	n
Lactose	−	D-Mannitol	w
Melibiose	+	D-Glucitol	+
Raffinose	−	α-Methyl-D-glucoside	−
Melezitose	w	Salicin	+
Inulin	−	D-Gluconate	−
Soluble starch	+	DL-Lactate	−
D-Xylose	+	Succinate	w
L-Arabinose	+	Citrate	−
D-Arabinose	−	Inositol	w
D-Ribose	−	Hexadecane	n
L-Rhamnose	+	Nitrate	−
D-Glucosamine	+	Vitamin-free	n

Additional assimilation tests and other growth characteristics:

Arbutin	+	Nitrite	−
L-Arabinitol	+	Ethylamine	+
D-Galacturonate	−	L-Lysine	+
Urease	−	Cadaverine	+
5% NaCl	+	Creatine	−
10% NaCl	−	Creatinine	−
10% $MgCl_2$	+	Starch formation	−
0.01% Cycloheximide	−	Growth at 30°C	+
DBB	−	Growth at 37°C	−

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: CBS 355.80, from oak wood infested with a bark beetle *(Hylecoetus dermestoides)*, Sweden, Francke-Grosmann.
Type strain: CBS 355.80.

Comments: The authentic strain no longer produced ascospores. It showed a remarkable dimorphism with subcultures easily segregating into restricted, purely yeastlike and expanding, hyphal colonies. Due to this dimorphism, physiological test results were often difficult to judge.

19.4. *Ascoidea rubescens* Brefeld (Brefeld & Lindau 1891)

Growth on 4% malt extract agar: After 10 days at 20–22°C, colonies are cream colored, glassy, thin, flat and expanding with a fimbriate margin. Budding cells are absent. Hyphae are 12–22 µm wide, thick-walled, branched at acute angles and produce ellipsoidal conidia singly or in sympodial order from terminal cells. Conidia are ellipsoidal with a truncate base, sessile, subhyaline, (15–20)×(23–38) µm.

Formation of ascospores: Asci are single, formed terminally on hyphae or lateral branchlets and are clavate, (25–30)×(100–150) µm, with thick walls. They contain 16–160 ascospores which are liberated by apical rupture of the ascus. Ascospores are ellipsoidal with a unilateral, gelatinous brim, (5–9)×(7–10) µm and cohere in slimy heads after liberation. New asci are formed percurrently 2 to 3 times inside the remains of a previous ascus; ascus remains are not retracted (Fig. 39). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	w	Methanol	−
L-Sorbose	−	Ethanol	+
Sucrose	+	Glycerol	w
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	n
Lactose	+	D-Mannitol	+
Melibiose	w	D-Glucitol	w
Raffinose	+	α-Methyl-D-glucoside	w
Melezitose	w	Salicin	−
Inulin	w	D-Gluconate	−
Soluble starch	+	DL-Lactate	−
D-Xylose	+	Succinate	−
L-Arabinose	−	Citrate	−
D-Arabinose	+	Inositol	w
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	−
D-Glucosamine	−	Vitamin-free	n

Additional assimilation tests and other growth characteristics:

Arbutin	w	Nitrite	–
L-Arabinitol	w	Ethylamine	+
D-Galacturonate	–	L-Lysine	+
Urease	–	Cadaverine	+
5% NaCl	–	Creatine	–
10% NaCl	–	Creatinine	–
5% $MgCl_2$	–	Starch formation	–
0.01% Cycloheximide	–	Growth at 30°C	+
DBB	–	Growth at 37°C	–

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 116.35, Fitzpatrick; CBS 111.48, from tree, Varitchak, Yugoslavia.

Type strain: A living ex-type culture is not available.

Comments: Extensive descriptions and illustrations of the species were given by Walker (1931) and Batra (1978). All cells of the thallus, including conidia, are multinucleate, with the exception of ascospores, which are uninucleate (Walker 1935). Ascogenesis was described and illustrated by Popta (1899). The species is easily recognized by its conidial dimensions and by conidia being non-catenate and lacking budding cells. *A. rubescens* grows very poorly in submersion, which hampered interpretation of physiological test results.

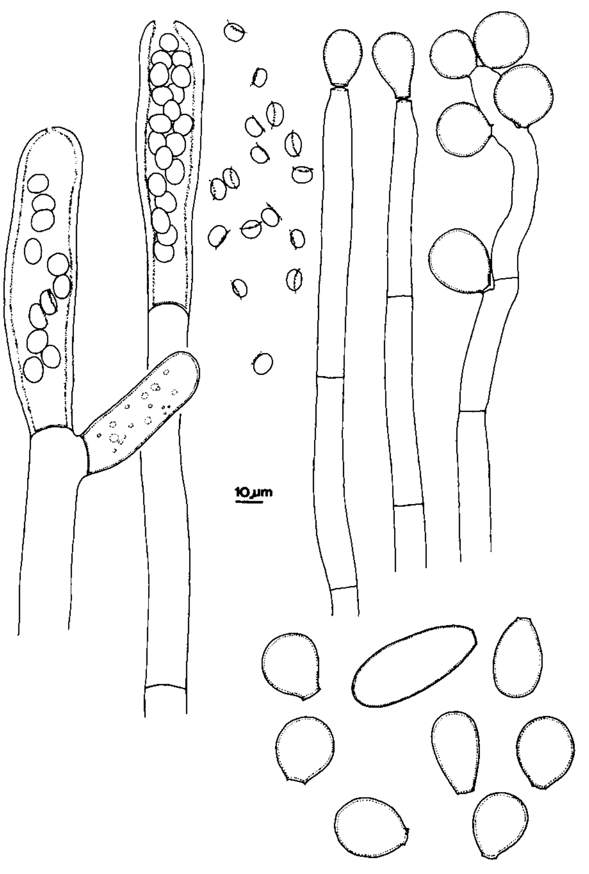


Fig. 39. *A. rubescens*, CBS 111.48. Asci, opening at the apex and liberating ascospores. True hyphae with sympodial conidia; liberated conidia with truncate bases. Malt extract agar, 22°C, 2 months.

Comments on the genus

Ascoidea species live in close association with bark beetles, in slime fluxes of trees or behind bark. All species appear primarily disseminated by insect vectors (Batra 1963b). Hyphal septa are perforated by micropores (von Arx and van der Walt 1986).

No specimen of *A. asiatica* Batra & Francke-Grosmann (1964) was available for study; live cultures of this species may be lost (L.R. Batra, personal communication).

Ascoidea saprolegnioides Holtermann (1898) is not known in culture. It would differ from the remaining *Ascoidea* species in having spherical ascospores.

20. *Babjevia* van der Walt & M.Th. Smith

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is by multilateral budding, mainly on a broad base. The cells are spherical, ovoidal, or occasionally elongated. Short, septate hyphae may be formed. Septa have a single, open, central pore. Cultures grow slowly.

Asci are saccate, attached to another cell, and produce one to many spores. Ascospores are hyaline, spherical to ovoidal, smooth and with a single layered wall.

Sugars are not fermented. Imidazole is utilized as source of nitrogen, but not nitrate. Diazonium blue B reaction is negative.

Type species

Babjevia anomala (Bab'eva & Gorin) van der Walt & M.Th. Smith

Species accepted

1. *Babjevia anomala* (Bab'eva & Gorin) van der Walt & M.Th. Smith (1995)

Systematic discussion of the species

20.1. *Babjevia anomala* (Bab'eva & Gorin) van der Walt & M.Th. Smith (Smith et al. 1995b)

Synonym:
Lipomyces anomalus Bab'eva & Gorin (1975).

Growth in malt extract: Growth is very slow. After 10 days at 20°C, the cells are spheroidal or ovoidal, (4.3–7.0)×(5.4–9.0) µm. A rudimentary pseudomycelium is formed. After 1 month at 20°, a small amount of sediment is present.

Growth on malt agar: Growth is slow and becomes visible only after one week at 20°C. After 10 days at 18°C, vegetative reproduction is mainly by budding on a broad base and the cells occur mostly as pseudohyphae. The cells are spheroidal or ovoidal, (4.5–7.0)×(5.3–9.0) µm and occasionally elongated, (4.0–10.0)×(8.0–18.0) µm. Capsules are not formed. Young cells usually contain one large vacuole and several small dark granules. After one month at 20°C, the streak culture is cream-colored, mat, and corrugated. Large spheroidal or ovoidal cells with many buds are common.

Dalmau plate cultures on potato agar: A simple pseudomycelium consisting of rows of cells formed by bud-fission is abundantly formed (Fig. 40).

Formation of ascospores: In the original description the generative life-cycle is described as follows. The species is homothallic. Asci are usually formed by conjugation of two buds on the same cell or on different cells. The conjugating buds are always separated from the parent cells by septa. This process closely resembles gametogangiogamy. After conjugation, the larger bud may separate from the parent cell. Several sac-like asci may be formed on the same cell (Fig. 40). Sometimes ascospores are formed within pseudomycelial cells or in cells with a great number of buds. The number of spores per ascus varies from 4 to 30 or more. The three strains investigated produced 1–4 ascospores; these are spheroidal, rarely ovoidal, and with one or two small droplets. The surface of the spores is smooth.

Ascosporulation was observed after 1–2 months incubation on diluted (1/10 or 1/20) V8 or YM agars at low temperatures (12–17°C).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	–
Sucrose	–	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	+	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Galactonate	–	Growth at 25°	+
0.1% Cycloheximide	+	Growth at 30°	–
Starch formation	w		

Co-Q: 9 (Yamada et al. 1986c, Billon-Grand 1987)

Mol% G+C: 43.8, CBS 6740 (BD: Phaff and Holzschu, in Phaff and Kurtzman 1984); 41.7–44.9, 3 strains (T_m: Smith, unpublished)

Origin of the strains studied: CBS 6740 (ATCC 32435; NRRL Y-7931; VKM Y-1969), type strain, podzolic soil, Armenia, I.P. Bab'eva; CBS 7606 (VKM Y-2716), V.I. Golubev; CBS 7607 (KBP-2593), USSR, I. Chernov.

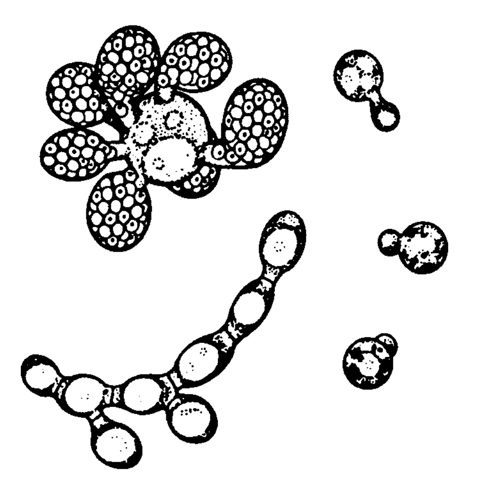


Fig. 40. *B. anomala*. Budding cells, pseudohyphae and a cell with multispored asci. (After Bab'eva and Gorin 1975; from Phaff and Kurtzman 1984).

Type strain: CBS 6740, isolated by Bab'eva and Gorin (1975).

Comments on the genus

During a study of yeasts associated with podzolic soils of the northern Taiga zone, Bab'eva and Gorin (1975) recovered strains which formed multispored, saccate asci borne on other cells. Regarding these isolates as representatives of the genus *Lipomyces,* they assigned them to a new taxon, *L. anomalus,* based on differences with the type species *L. starkeyi.* Major characteristics of *L. anomalus* included formation of non-encapsulated cells, pseudohyphal cell aggregates, pulvinate colonies on solid substrates, lower optimal growth temperature and utilization of relatively few carbon sources. Kurtzman and Liu (1990) and Yamada and Nogawa (1990b) compared partial sequences of large and small subunit rRNAs from species of *Lipomyces* and concluded that *L. anomalus* represented a divergent member of *Lipomyces*. Based on these analyses and reexamination of the fine structure of septa and ascosporal walls, Smith et al. (1995b) transferred *L. anomalus* to the new genus *Babjevia*.

21. *Cephaloascus* Hanawa

G.S. de Hoog and C.P. Kurtzman

Diagnosis of the genus

Colonies are smooth or farinose, and white to pale cream-colored. Budding cells may be present; pseudomycelium and true hyphae are prevalent. Conidia are formed sympodially on large denticles, in short chains; ultimate conidia are clavate.

Ascophores are erect, hyaline or locally brown, and bear a cluster of asci at their apex. Asci arise in short, branched chains, ellipsoidal, thin-walled, and open by apical deterioration; asci mostly contain 4 hat-shaped ascospores.

Sugars may be weakly fermented. Nitrate is not assimilated. Diazonium blue B reaction is negative.

Type species

Cephaloascus fragrans Hanawa

Species accepted

1. *Cephaloascus albidus* Kurtzman (1977)
2. *Cephaloascus fragrans* Hanawa (1920)

Key to species

See Table 18.

1. a Melibiose assimilated; ascophores without metulae, bearing branched chains of asci .... *C. albidus*: p. 143
   b Melibiose not assimilated; ascophores bearing whorls of metulae with asci .... *C. fragrans*: p. 144

Table 18
Key characters of species in the genus *Cephaloascus*

Species	Glucose fermentation	Assimilation			Mating system
		Melibiose	Raffinose	Salicin	
Cephaloascus albidus	w	+	+	+	Heterothallic
C. fragrans	–	–	–	–	Homothallic

Systematic discussion of the species

21.1. *Cephaloascus albidus* Kurtzman (1977)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies attain 10 mm diameter, are slimy, hyaline at the center, with a soft texture. The teleomorph is formed in the dry, farinose, snow-white expanding zone. Margins are straight. Hyphae are 2–3 µm wide, hyaline, profusely branched, and bear apical clusters of conidia which are sessile or on marked denticles; the conidial apparatus easily falls apart into separate cells. Conidia are ellipsoidal to clavate, (1.5–2.5)×(3–5) µm.

Growth on the surface of assimilation media: A heavy, dry pellicle is formed.

Formation of ascospores: Ascophores are erect, stout, hyaline, smooth and firm-walled, (5–7) µm wide, slightly tapering towards the tip, and up to 200 µm high. Asci are formed in short chains in a terminal cluster on the ascophore, are thin-walled, and ellipsoidal, (2.5–3.0)×(5–7) µm; occasionally they are formed on undifferentiated hyphae. Asci mostly contain 4 ascospores which are hat-shaped, (1.5–2.0)×(2.5–3.0) µm; asci open by apical deterioration (Fig. 41). The species is heterothallic.

Fermentation:

Glucose	w	Lactose	–
Galactose	w	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+/w	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+/w
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

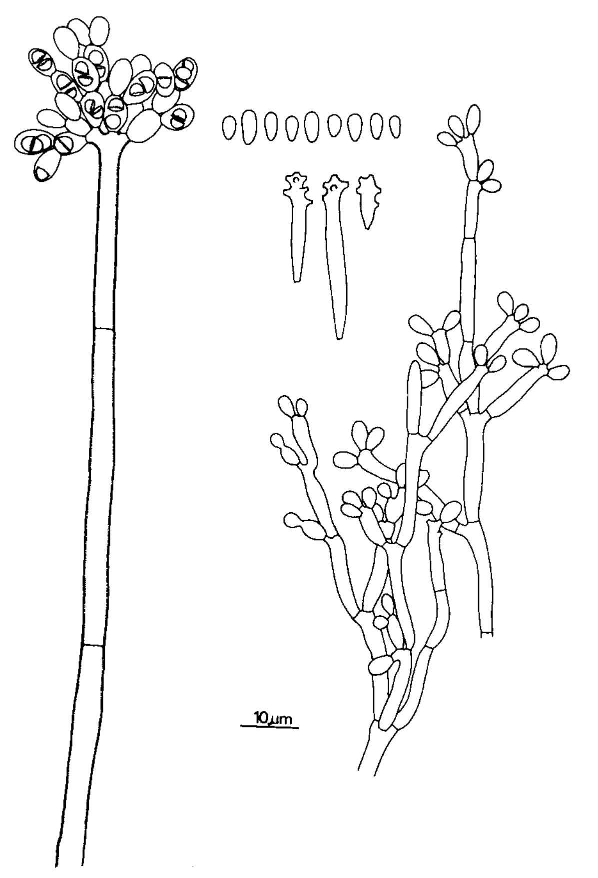


Fig. 41. *C. albidus.* Ascophore apex with branched chains of asci, CBS 390.77×391.77; anamorph composed of branched hyphae, liberated terminal conidia and larger, lower conidia with large denticles, CBS 390.77. 4% Malt extract/0.5% yeast extract agar, 3 weeks.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	10% NaCl/5% glucose	+
Saccharate	–	Starch formation	–
Cadaverine	+	Gelatin liquefaction	–
Creatine	–	Nitrite	–
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

Co-Q: 9, CBS 389.77 (Yamada et al. 1987b).
Mol% G+C: 37.8, CBS 389.77 (BD: Kurtzman, unpublished).

Origin of the strain studied: CBS 389.77 (NRRL Y-7343), from cranberry pumace, Canada.

Complementary mating types: CBS 390.77 (NRRL Y-7343-2) and 391.77 (NRRL Y-7343-6), obtained as single-ascospore isolates from the diploid type strain.

Type strain: CBS 389.77, from cranberry pumace.

Comments: The species was described in detail by Kurtzman (1977). Wang and Zabel (1990) described several strains from treated pine poles, which were identified as *C. albidus* on the basis of hyaline conidiophores. However, the difference in pigmentation of ascophores in the two species is unreliable as a specific criterion (Batra 1963c). Judging from the absence of growth with raffinose, these strains were probably *C. fragrans,* which is known as a common inhabitant of pine wood.

Cephaloascus albidus has septa with a central micropore. This is in contrast to *C. fragrans,* which has a simple pore without Woronin bodies but with numerous electron-dense circular bodies (Kurtzman 1977).

21.2. *Cephaloascus fragrans* Hanawa (1920)

Synonyms:
Ascocybe grovesii Wells (1954)
Aureomyces mirabilis Ruokola & Salonen (1970)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies attain 10 mm diameter, are slimy, pale cream-colored, dry and become pale brown due to production of the teleomorph. Cultures with the teleomorph often exude a brown pigment into the medium. The margin is straight. Hyphae are 1–2 μm wide, hyaline, profusely branched, and apically bear clusters of conidia which are sessile or on marked denticles; the conidial apparatus easily falls apart into separate cells. Conidia are ellipsoidal, (1.5–2.5)×(4–6) μm when terminal, or much longer when derived from disarticulating hyphae.

Growth on the surface of assimilation media: A dry, climbing pellicle is formed.

Formation of ascospores: Ascophores are erect, stout, thick-walled, often become rough-walled and golden brown in the apical part, about 8 μm wide at the base, tapering to (4–6) μm just below the slightly swollen apex, and up to 500 μm high. Asci are formed in short chains on metulae at the apex of ascophores, thin-walled, ellipsoidal, (3–4)×(5–7) μm, and open by deterioration at the tip (Fig. 42). Asci contain 2–4 ascospores which are hat-shaped, (1.8–2.5)×(2–3) μm. The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	v
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	w/–
D-Xylose	+	Succinate	+/w
L-Arabinose	+	Citrate	+/w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	10% NaCl/5% glucose	w/–
Saccharate	–	Starch formation	–
Cadaverine	+	Gelatin liquefaction	–
Creatine	–	Nitrite	–
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

Co-Q: 9, CBS 121.29 and 5 additional strains (Yamada et al. 1987b).
Mol% G+C: 39.9, CBS 121.29 (BD: Kurtzman, unpublished).

Origin of the strains studied: CBS 121.29 (ATCC 36174, NRRL Y-6742), from human ear, Hanawa; CBS 150.61 (ATCC 12091, DAOM 33766), type strain of *Ascocybe grovesii,* from pine (*Pinus* sp.) wood, Wells, Canada; CBS 183.71 (ATCC 22396), type strain of *Aureomyces mirabilis,* from pine (*Pinus* sp.) wood, Ruokola, Finland; from pine (*Pinus* sp.) wood (1); from oak (*Quercus* sp.) wood (1).

Type strain: CBS 121.29, from a human ear.

Comments: The species was originally isolated from a human ear, but its prevalent ecological niche seems to be pine or oak wood in association with bark insects. An extended description of the species was given by Schippers-Lammertse and Heyting (1962).

The teleomorph is abundantly produced on nutritionally poor media. Cultures with teleomorphs often exude a brown, water-soluble pigment into the medium. The pigment was produced by nearly all strains in growth tests with ethylamine, lysine and cadaverine, but remained absent with most carbon compounds tested.

Kurtzman (1977) found the species to have simple septal pores without Woronin bodies but with numerous, electron-dense circular bodies. The species is diploid (Wilson 1961). The cell walls are rich in mannose, while cellulose and rhamnose are absent (Weijman 1976).

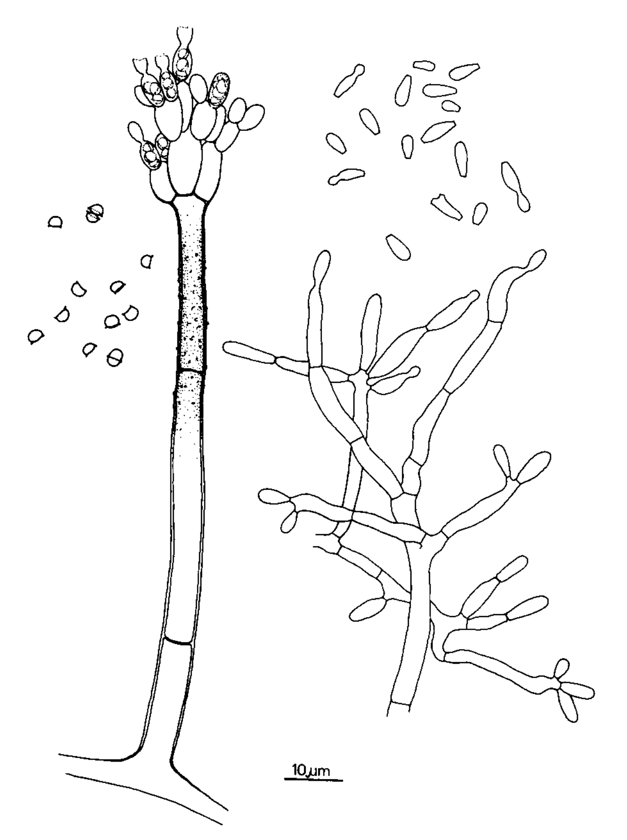


Fig. 42. *C. fragrans.* Ascophores with sterile metulae bearing chains of asci which deteriorate at the distal end, CBS 150.61, 5% malt extract agar, 25°C, 1 week; hyphae with conidia, CBS 121.29.

22. *Citeromyces* Santa María

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal to ellipsoidal. Neither pseudohyphae nor true hyphae are produced.

Asci are persistent and contain one or, infrequently, two roughened spheroidal ascospores. Asci arise from diploid cells or from conjugation between complementary mating types.

Sugars are fermented. Nitrate is assimilated. Coenzyme Q-8 is present. Diazonium blue B reaction is negative.

Type species

Citeromyces matritensis (Santa María) Santa María

Species accepted

1. *Citeromyces matritensis* (Santa María) Santa María (1957)

Systematic discussion of the species

22.1. *Citeromyces matritensis* (Santa María) Santa María (1957)

Anamorph: *Candida globosa* Yarrow & S.A. Meyer

Synonyms:

Hansenula matritensis Santa María (1956a)

Torula globosa Olson & Hammer (1935) nom. nud.

Torulopsis globosa (Olson & Hammer) Lodder & Kreger-van Rij (1952) nom. nud.

Candida globosa Yarrow & S.A. Meyer (1978)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal (3.0–9.5)×(4.0–10.5) µm, occur as singles, pairs, or in threes, and may have one or two buds. Growth is butyrous and white to faintly tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass lacks pseudohyphae or true hyphae. Aerobic growth is butyrous, cream colored and slightly glistening with fine striations and a tannish, depressed center. Colony margins are entire or finely lobed.

Formation of ascospores: Many natural isolates are diploid and the vegetative cells convert directly into asci. Asci have thick persistent walls and contain one or, infrequently, two spheroidal ascospores with a roughened wall and a prominent lipid droplet (Fig. 43). Sporulation is usually abundant on YM agar, and well-sporulated cultures turn red to brown. Wickerham (1958) demonstrated *C. matritensis* to be heterothallic by heat treatment of a sporogenous culture of the type strain. In addition, he showed that the type strain of *Candida (Torulopsis) globosa* (NRRL Y-1506, CBS 162) conjugated with one of the mating types (NRRL Y-2407-9), but apparently represents a unisexual diploid form of this species because it also sporulated alone on media with high sugar concentrations. Complementary mating types show a strong sexual agglutination reaction if grown in liquid medium rather than on an agar medium (Wickerham 1958).

Ascospores were observed on YM-, malt extract-, and V8 agars after 10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w
Sucrose	+/w	Trehalose	w/–
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+/w
Sucrose	+	Glycerol	+/w
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	–
Inulin	v	D-Gluconate	+
Soluble starch	–	DL-Lactate	w/–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	v	Vitamin-free	+/w

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 8 (Yamada et al. 1973a).

Mol% G+C: 45.5, 45.2, CBS 2764 (T_m: Nakase and Komagata 1968a; BD: C.P. Kurtzman, unpublished).

Origin of the strains studied: NRRL Y-2407 (CBS 2764), fermenting fruit in syrup, J. Santa María, Spain (1); sugar, J. Santa María, Spain (1); condensed milk from the Netherlands (1), Spain (1); NRRL Y-1506 (CBS 162), type strain of *Candida (Torulopsis) globosa*, from condensed milk, U.S.A.; flux from sandalwood *(Myoporum sandwicense)*, Hawaii, U.S.A. (5).

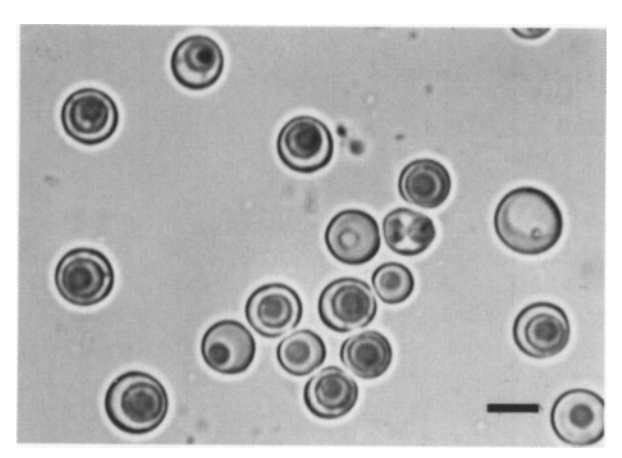


Fig. 43. *C. matritensis,* CBS 2764. Asci with ascospores, after 10 days, YM agar, 25°C. Bar = 5 µm.

Complementary mating types: NRRL Y-2407-9 (CBS 7103) and NRRL Y-2407-10 (CBS 7104), isolated following heat treatment of the diploid type strain.

Type strain: CBS 2764 (NRRL Y-2407) received from J. Santa María as the type strain of *Hansenula matritensis.* This strain was isolated from fruit preserved in syrup.

Comments on the genus

Citeromyces is unusual among taxa that form rugose spheroidal ascospores because it is characterized by coenzyme Q-8. Whether *Citeromyces* represents a unique phylogenetic line is unknown and, consequently, knowledge of its position among the yeasts must await future molecular comparisons.

Original isolates of *C. matritensis* were from foods with high sugar content. More recently, the occurrence of this species has been documented in nature with repeated isolations from fluxes of the sandalwood tree (H.J. Phaff and W.T. Starmer, personal communication).

23. *Clavispora* Rodrigues de Miranda

M.A. Lachance and H.J. Phaff

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are ovoid, ellipsoidal or elongate. Pseudomycelium may be formed, but true mycelium is not formed.

Conjugation of haploid cells of opposite mating types precedes ascus formation. The ascospores are clavate, sometimes containing a small oil droplet; one or two (rarely three or four) ascospores are formed per ascus. The spore wall may have small warts visible by electron microscopy. The spores are easily liberated from the ascus.

Glucose is fermented. Nitrate is not assimilated. Coenzyme Q-8. Diazonium blue B reaction is negative.

Type species

Clavispora lusitaniae Rodrigues de Miranda

Species accepted

1. *Clavispora lusitaniae* Rodrigues de Miranda (1979)
2. *Clavispora opuntiae* Phaff, Miranda, Starmer, Tredick & Barker (1986)

Key to species

See Table 19.

1. a L-Rhamnose or L-lysine assimilated, growth in the presence of cycloheximide 10 mg/l .... *C. lusitaniae*: p. 148
   b L-Rhamnose and L-lysine not assimilated, no growth in the presence of cycloheximide 10 mg/l .... *C. opuntiae*: p. 150

Table 19
Key characters of species in the genus *Clavispora*

Species	Assimilation			Cycloheximide 10 mg/l
	L-Rhamnose	Hexadecane	L-Lysine [a]	
Clavispora lusitaniae	v	w/–	+	+/w
C. opuntiae	–	+	w/–	–

[a] As sole nitrogen source.

Systematic discussion of the species

23.1. *Clavispora lusitaniae* Rodrigues de Miranda (1979)

Anamorph: *Candida lusitaniae* van Uden & do Carmo-Sousa
Synonyms:
Candida lusitaniae van Uden & do Carmo-Sousa (1959)
Candida parapsilosis (Ashford) Langeron & Talice var. *obtusa* Dietrichson (1954) nom. nud.
Candida obtusa (Dietrichson) van Uden & do Carmo-Sousa ex van Uden & H.R. Buckley (1970)
Saccharomyces carmosousae Montrocher (1967)

Growth on YM agar: After 3 days at 25°C, the cells are subglobose, ovoidal, to elongate, (2–6)×(3–10) µm, single, in pairs or in short chains. Growth is butyrous, white to cream colored, glistening or occasionally dull and rugose.

Growth in glucose–yeast extract broth: A sediment is normally formed after 2 weeks at 25°C. Occasionally growth may be flocculent.

Dalmau plate culture on corn meal agar: After one week at 25°C, pseudomycelium is abundant and well developed. Colonies may be fringed with pseudohyphae.

Formation of ascospores: Asci are bilobate, containing usually one or two (rarely three or four) clavate ascospores (Fig. 44). Warts may (Rodrigues de Miranda 1984a) or may not (Rodrigues de Miranda 1979) be visible by electron microscopy. The ascospores are liberated from the asci soon after their formation. On rare occasions, spherical ascospores are formed.

Abundant sporulation occurs 2–4 days at 17–25°C after mixing cultures of compatible mating types on 1% malt extract agar. Nearly all isolates are fertile.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	–
Sucrose	v	Trehalose	v
Maltose	v		

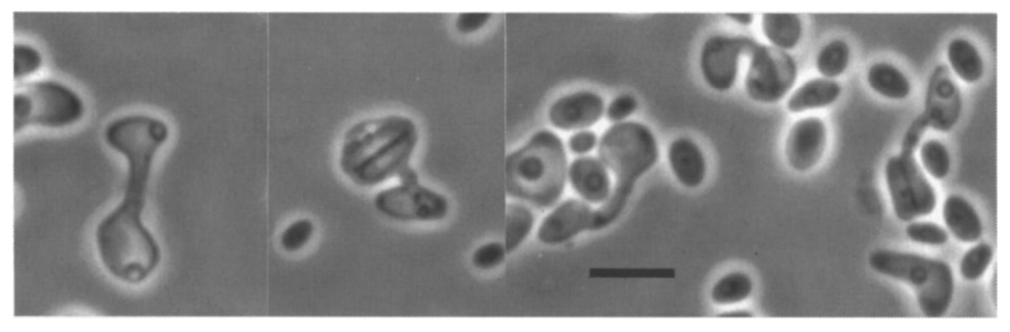


Fig. 44. *C. lusitaniae*, CBS 4413×CBS 6936. Conjugating cells and asci after 4 days on 1% malt extract agar, 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	+/w
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	w/–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Cycloheximide 10 mg/l	+/w
Ethylamine.HCL	+	Gelatin liquefaction	–
L-Lysine	+	Growth at 37°C	+
Glucose 50%	s/w		

Co-Q: 8 (Yamada and Kondo 1973).

Mol% G+C: 45.1–45.7, 5 strains, including CBS 4413 and CBS 6936 (BD: Lachance et al. 1986).

Origin of the strains studied: CBS 1944, clinical specimen, Dietrichson, Norway, type strain of *Candida obtusa;* CBS 4413, type strain of *Candida lusitaniae,* and CBS 4415, from pig digestive tract, van Uden, Portugal; CBS 6936, from peel juice of *Citrus,* Mateles, Israel; CBS 7270, received by CBS as *Candida kruisii,* clinical specimen, Woodgyer, New Zealand. SU(B)79-257.1, from trunk rot of boojum tree (*Idria columnaris* Kellogg), and SU(B)79-267.12 and SU(B)86-460.5, from stem rot of agria cactus, [*Stenocereus gummosus* (Engelmann) Gibson & Horak], Baja California N.; SU(B)80-29, from leaf rot of *Agave* L. sp., Arizona; SU(B)86-287, from rotting tissue of cactus, [*Cephalocereus russelianus,* (Otto) Rose], Venezuela; SU(B)86-780.2, from stem rot of cactus [*Cephalocereus hoppenstedtii* (Weber) K. Schum.] Mexico; SU(B)86-968.1, from cladode rot of prickly pear [*Opuntia ficus-indica* (L.) Miller] Florida, U.S.A., Starmer; UCD-FST 81-467C, from cladode rot of prickly pear, (*Opuntia phaeacantha* Engelmann), Arizona, U.S.A.; UWO(PS)83-1068.1 and UWO(PS)83-1080.1, from decaying fruit of prickly pear (*Opuntia stricta* Haworth) and UWO(PS)83-1156.2, from fly (*Drosophila bromeliae* Sturtevant) collected on morning glory (*Ipomoea* L. sp.), Cayman Islands; UWO(PS)85-286.1, from fly (*Drosophila carbonaria* Patterson & Wheeler) collected on mesquite [*Prosopis juliflora* Swartz (DC)], Arizona, U.S.A. and UWO(PS)WN9-8 and UWO(PS)WN9-20, from effluents of chocolate factory, Ontario, Canada.

Complementary mating types: CBS 6936 (h^+) and CBS 4413 (h^-).

Type strain: CBS 6936, isolated by Mateles from citrus peel juice; strain CBS 4413, isolated by van Uden from a pig is the isotype.

Comments: It was initially thought (Rodrigues de Miranda 1984a; Lachance et al. 1986) that *C. lusitaniae* consistently assimilates L-rhamnose, suggesting that this response could serve as a diagnostic species character. Some strains examined more recently, CBS 7270 and SU(B)86-287, failed to utilize L-rhamnose, but their identity was ascertained by mating experiments. An imperfect positive correlation was found between L-rhamnose utilization and that of citric acid in this case. In a study of 13 clinical isolates, Gargeya et al. (1990) found two strains that neither utilized L-rhamnose nor exhibited mating reactions. DNA reassociation, however, demonstrated a high degree of genetic relatedness between these variants and authentic *C. lusitaniae* strains. Also, one of their mating strains failed to utilize maltose. Rodrigues de Miranda (1984a) reported that *C. lusitaniae* exhibits vitamin independence. None of the strains we have examined grew on vitamin-free medium. The 11 strains studied by van Uden and Buckley (1970) and the 10 strains examined by Barnett et al. (1983) required biotin, pyridoxine, and thiamin for growth.

Mating compatibility among strains of *C. lusitaniae* generally follows a pattern clearly consistent with a single-locus, biallelic system. The holotype (CBS 6936) has been arbitrarily designated h^+, and the isotype (CBS 4413), h^- (Lachance et al. 1986). According to Gargeya et al. (1990), h^+ corresponds to the designation *a* used by other authors (and accordingly h^- corresponds to α), although the initial assignment

of mating types is not clear due to a typographical error in Rodrigues de Miranda's (1979) original description. Strains CBS 1944, CBS 7270, SU(B)79-257.1, SU(B)80-29, and UWO(PS)WN9-8 are of mating type h^+, while CBS 4415, SU(B)79-267.12, SU(B)86-460.5, SU(B)86-287, SU(B)86-780.2, SU(B)86-968.1, UCD-FST 81-467C, UWO(PS)83-1068.1, UWO(PS)-83-1080.1, UWO(PS)83-1156.2, UWO(PS)85-286.1, and UWO(PS)WN9-20 are of mating type h^-.

The ecological niche of *C. lusitaniae* is ill-defined. The yeast is recovered quite frequently in clinical specimens, but is not regarded as a true human pathogen (Hurley et al. 1987). It was first recognized as an opportunistic infectious organism by Holzschu et al. (1979), and has since turned up in over a hundred specimens from patients suffering from immune deficiencies (Gargeya et al. 1990). A study of 35 clinical isolates (Merz et al. 1992) revealed that a significant amount of genetic variation occurs from clone to clone. These could be assigned, on the basis of their electrokaryotypes, to 15 groups corresponding to the 15 patients from which they had been isolated. Although *C. lusitaniae* occurs in cactus, it cannot be considered cactophilic, having been reported only 10 times in nearly two thousand samples (Starmer et al. 1990). Its ability to grow at elevated temperatures combined with its recovery in several samples from warm-blooded animals (van Uden and Buckley 1970) may provide clues on the nature of its natural habitat, but alternatively may reflect the greater intensity of research efforts in that area in the past.

23.2. *Clavispora opuntiae* Phaff, Miranda, Starmer, Tredick & Barker (1986)

Synonym:

Lodderomyces opuntiae Starmer (1981) nom. nud.

Growth on YM agar: After 3 days at 25°C, the cells are subglobose, ovoidal to elongate (2–5)×(3–16) μm, single, in pairs or in short chains. Growth is butyrous, white to cream colored and glistening. A characteristic fringe of submerged pseudomycelium is formed. After storage on YM agar slants covered with mineral oil for several months, growth on YM agar may take the form of rugose, convoluted colonies.

Growth in glucose–yeast extract broth: A sediment is normally formed after 2 weeks at 25°C. After storage on YM agar slants covered with mineral oil for several months, a heavy pellicle may be formed on liquid media after a week.

Dalmau plate culture on corn meal agar: After one week at 25°C, pseudomycelium is abundant and well developed. Colonies are fringed with pseudohyphae.

Formation of ascospores: Asci are bilobate, containing usually one or two (rarely three or four) clavate ascospores similar to those formed by *C. lusitaniae* (Fig. 44). Warts are visible by electron microscopy only (Phaff et al. 1986). The ascospores are liberated from the asci soon after their formation.

In most strains, abundant sporulation occurs 2–4 days at 17–25°C after mixing compatible mating types on 1% malt extract agar.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	+	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	s
L-Rhamnose	–	Nitrate	–
D-Glucosamine	w/–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Cycloheximide 10 mg/l	–
Ethylamine.HCL	+	Gelatin liquefaction	–
L-Lysine	w/–	Growth at 37°C	+
Glucose 50%	–		

Co-Q: 8 (Phaff et al. 1986).

Mol% G+C: 43.1–44.1, 11 strains, including UCD-FST 77-279 (CBS 7068) and UCD-FST 78-540A (CBS 7069) (BD: Phaff et al. 1986).

Origin of the strains studied: UCD-FST 77-279 (CBS 7068) and UCD-FST 78-540A (CBS 7069) are among hundreds of strains isolated from various species of prickly pear: in Australia, *Opuntia vulgaris* Miller (syn. *O. monacantha*), *O. stricta* Haworth, *O. streptacantha* Lemaire, *O. tomentosa* Salm-Dyck; in Venezuela, *O. boldinghii* Britton & Rose, *O. elatior* Miller; in Hawaii, *O. megacantha* Salm-Dyck (syn. *O. ficus-indica*); in Peru, *Opuntia* (Tournefort) Miller sp.; in Arizona, U.S.A., *O. ficus-indica, O. phaeacantha*; in Texas, *O. lindheimeri* Engelmann; in the Bahamas, the Antilles, and the Caribbean, *O. moniliformis* (L.) Haworth, *O. stricta* [UWO(PS)83-877.1 (ATCC60725)]; in Mexico, *O. ficus-indica, O. oricola* Philbrick, *O. wilcoxii* Britton & Rose, *Nopalea* Salm-Dyck sp. (syn. *Opuntia* sp.); in Spain and South Africa, *O. ficus-indica;* in Brazil, *O. vulgaris* (received from Rosa). Some strains are from columnar cactus species, *Stenocereus alamosensis* (Coulter) Gibson & Horak, *S. griseus* (Hawthorn) Buxbaum, *S. hystrix* (Hawthorn) Buxbaum, *S. pruinosus* (Otto) Buxbaum, *S. stellatus* (Pfeiffer) Riccobono, *Cephalocereus royenii* (Linnaeus) Britton & Rose [UWO(PS)-718.1], *C. lanuginosus* (L.) Britton & Rose, *Myrtillocactus cochal* (Orcutt) Britton & Rose. A few strains are from cactus flies:

Drosophila mettleri Heed and *D. nigrospiracula* Patterson & Wheeler recovered on giant saguaro cactus [*Carnegiea gigantea* (Engelmann) Britton & Rose], Arizona, U.S.A.; *D. mulleri* Sturtevant [UWO(PS)83-803.2 (ATCC 60724)] and *D. mayaguana* Vilela collected on *Opuntia* L. spp., Caribbean. Several strains were recovered from the prickly pear moth, *Cactoblastis cactorum* Berg. Some strains (received from Rosa) were recovered from feeding sites of the moth *Sigelgaita* (Heirich) sp. in *Pilosocereus arrabidae* (Lem.) Byles et Rowl. in Brazil. Strain UWO(PS)85-351.1 is from the exudate of an oak (*Quercus emoryi* Torr.) growing in the vicinity of cacti in Arizona.

Complementary mating types: CBS 7068 (h^+) and CBS 7069 (h^-).

Type strain: CBS 7068; strain CBS 7069 is the isotype. Both are prickly pear isolates from Australia.

Comments: *C. opuntiae* was described (Phaff et al. 1986) to accommodate isolates consistently recovered from prickly pear (*Opuntia* L. spp.), and less frequently from other cacti. Although *C. opuntiae* occurs in decaying cactus fruit as well as somatic tissue, its host specificity is remarkable, in view of its worldwide distribution. Found in 10% of all prickly pear samples ever collected (Lachance et al. 1988), *C. opuntiae* accounts for 5% of the yeasts isolated from prickly pears and 2% or less of isolates from other cactus species (Starmer et al. 1990). Many vectors (e.g., *Drosophila* spp.) are likely to contribute significantly to the yeast's distribution, but the prickly pear moth, *Cactoblastis cactorum* Berg, is thought to play a major role in confining *C. opuntiae* mostly to *Opuntia* spp., as the occurrence of the moth coincides to some extent with the abundance of the yeast (Starmer et al. 1988a). DNA fingerprinting (Lachance 1990a) indicated that cacti may bear, in their *C. opuntiae* biota, a genetically diverse resident component as well as a more homogeneous population associated with *C. cactorum.* Rosa et al. (1992), working in the region of Rio de Janeiro, Brazil, reported that *C. opuntiae* occurs there less abundantly in *Opuntia* species than in the columnar cactus *Pilosocereus arrabidae* (Lem.) Byles & Rowl., where the yeast appears to exist in association with the moth *Sigelgaita* (Heirich) sp.

Mating compatibility in *C. opuntiae* is typically a single-locus, biallelic system. The holotype (CBS 7068) is designated h^+, and the isotype (CBS 7069), h^- (Phaff et al. 1986). Considerable inequalities are observed in the distribution of mating types within local populations (Lachance 1990a). An h^+:h^- ratio greater than 30:1 is observed among Australian isolates (Phaff et al. 1986), with the nearly exact reverse in strains collected on the Bahamian Island of Great Inagua (M.A. Lachance, H.J. Phaff and W.T. Starmer, unpublished data). Brazilian isolates exhibited a 20:1 mating type ratio (Rosa et al. 1992). Even more extreme disparities are known to exist on islands of the Hawaiian archipelago (M.A. Lachance and W.T. Starmer, unpublished data).

Although some strains of *C. opuntiae* may exhibit delayed reactions for the assimilation of trehalose and a few other carbon compounds, variation is rather minimal in this species. The exception is strain UWO(PS)83-718.1 (Lachance et al. 1986), which differs from others by its very weak growth on 2-keto-D-gluconate and its higher sensitivity to inhibition by saponins of the agria cactus, *Stenocereus gummosus* (Englemann) Gibson & Horak. These differences are paralleled by the fact that this is the only strain of *C. opuntiae* ever recovered from *Cephalocereus royenii* (L.) Britton & Rose, even though nearly 100 plants of that cactus have been sampled (Starmer et al. 1990). Matings between strain UWO(PS)83-718.1 (h^-) and several h^+ strains invariably lead to very low meiotic recombination (P. Lo and M.A. Lachance, unpublished data). In addition, the large subunit rDNA coding region of that strain contains a unique restriction site not found in the rest of the species (Lachance 1990a). While it would be premature to describe a variety on the basis of a single strain, the anticipated recovery of additional strains from *Cephalocereus royenii* or from hosts sympatric to that cactus is expected to provide insight on trans-specific evolution in this yeast species.

C. opuntiae is capable of ethanol production from D-xylose under aerobic conditions (Nigam et al. 1985). Of 11 strains studied, UWO(PS)83-803.2 (ATCC60724) and UWO(PS)83-877.1 (ATCC60725) had the highest yields of 34 and 57% of the theoretical value under certain conditions.

Comments on the genus

Rodrigues de Miranda (1979) described the monotypic genus *Clavispora* after observing that strains previously assigned to *Candida lusitaniae* mated with a new strain isolated from *Citrus* peel juice by Mateles, in Israel, giving rise to clavate ascospores. The type strain of *Candida obtusa* (CBS 1944) was also found to be compatible. A strain isolated from a mushroom and designated as *Candida obtusa* var. *arabinosa* did not show sexual reactions with any of the mating types, and was reassigned to *Pichia mississippiensis* (Kurtzman et al. 1980a).

Prior to the publication of the genus *Clavispora* by Rodrigues de Miranda (1979), *C. opuntiae* was thought to represent a species of *Lodderomyces* because of the shape of its ascospores (e.g., Starmer 1981b). The morphological similarity of the new species with *C. lusitaniae* later became obvious and prompted the description by Phaff et al. (1986).

Although the two *Clavispora* species are barely distinguishable on the basis of physiology and morphology, they are sharply distinct at the genetic level. Without exception, mating is restricted to intraspecific crosses. DNA reassociation and rDNA restriction mapping clearly delineate the two taxa along the same boundaries (Lachance et al. 1986). Ecologically, a small amount of niche overlap is evident, *C. lusitaniae* being recovered on occasion

from prickly pear and *C. opuntiae* from columnar cacti. Because of its less diverse habitat, its slightly narrower nutritional range, its shorter rDNA repeat unit (7.6 as opposed to 9.0 kb), and its lower mol% G + C, *C. opuntiae* is viewed as the more evolutionarily derived of the two species.

The position of the genus *Clavispora* relative to other yeast genera is beginning to be clarified. Several other yeasts share DNA base compositions in the mid-40% values. Among those, certain *Metschnikowia* and *Pichia* species have physiological profiles that bear a superficial resemblance with those of *Clavispora* species. Only a few *Pichia* species share the Co-Q 8 characteristic. The ribosomal RNA sequencing studies of Barns et al. (1991) and Hendricks et al. (1991) together indicate that *Clavispora* (represented by *C. lusitaniae*) is phylogenetically distinct from *Candida albicans* and related *Candida* species, from *Candida guilliermondii,* from *Issatchenkia* species (represented by *Candida krusei*), from *Pichia* species (represented by *Pichia angusta* – syn. *Hansenula polymorpha*), from the moderately related species *Saccharomyces cerevisiae, Kluyveromyces lactis, Torulaspora delbrueckii* and *Candida glabrata,* or from *Yarrowia lipolytica.* Of all the yeasts compared in the distance matrix presented by Barns et al. (1991), *Candida guilliermondii* is the nearest neighbor to *Clavispora lusitaniae.*

Candida oleophila exhibits a remarkably high degree of phenetic similarity to *C. opuntiae,* has 42 mol% G + C (Nakase and Komagata 1971f), and could eventually be shown to have a sexual life cycle typical of *Clavispora.* Unlike *C. opuntiae* however, *Candida oleophila* grows vigorously on glucono-δ-lactone, utilizes L-lysine as sole nitrogen source, grows in the presence of 100 mg/l cycloheximide, and fails to grow at 37°C.

24. *Coccidiascus* Chatton emend. Lushbaugh, Rowton & McGhee

H.J. Phaff

Diagnosis of the genus

Vegetative cells spheroidal to ovoidal, 5–15 µm in length, with a large vacuole and nucleus. Vegetative reproduction by budding. Mycelium, pseudomycelium, or ballistospores are lacking. Vegetative cells, which are transformed into asci, elongate, forming banana-shaped to crescent-shaped asci arranged within parasitophorous vacuoles of *Drosophila* intestinal epithelium cells in the manner of coccidian schizonts. Thick-walled asci observed in host tissue range from 13–18 µm tip to tip. Mature asci contain two relatively thin-walled ascospores, closely intertwined in a helix. Asci from dead flies have been observed to contain up to eight ascospores. Ascospores extruded from asci are spindle-shaped, bilaterally flattened, without apical cytoplasmic appendages, each with an apical nucleus. Fermentation and assimilation reactions are unknown.

Type species

Coccidiascus legeri Chatton

Species accepted

1. *Coccidiascus legeri* Chatton (1913)

Systematic discussion of the species

24.1. *Coccidiascus legeri* Chatton (1913)

This organism has not been cultured and the description of this species therefore is the same as the generic description.

Type strain: Neotype material collected in *Drosophila melanogaster* intestine from Athens, Georgia, U.S.A., has been deposited in the New York Botanical Garden Cryptogamic Herbarium and in the Julian H. Miller Mycological Herbarium (GAM).

Comments on the genus

Chatton (1913) observed and named this species, which parasitized the intestinal cells of *Drosophila funebris* Fabr. in France. He was unable to cultivate it and as far as is known no type material was deposited in a herbarium. This species was rediscovered by Lushbaugh et al. (1976) as a parasite within vacuoles of the intestinal epithelium of *Drosophila melanogaster* Meigen in the state of Georgia, U.S.A. They provided an expanded description of *Coccidiascus legeri* illustrated by electron micrographs and light microscope photographs of material in situ (Fig. 45). They also were unable to culture the organism and, in the absence of a known holotype or lectotype, they designated a neotype which was deposited in the New York Botanical Garden Cryptogamic Herbarium (New York, U.S.A.).

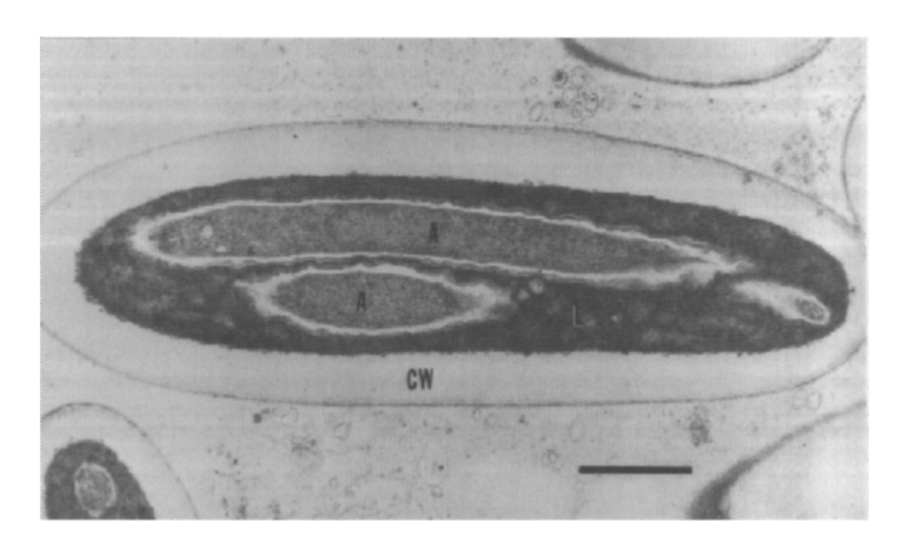


Fig. 45. *C. legeri.* Transmission electron micrograph of ascospores (A). One of the spores is shown nearly in its entirety, whereas only a portion of the second spore is seen. The ascus wall (CW) is noticeably thickened (from Lushbaugh et al. 1976). Bar = 1 µm.

25. *Cyniclomyces* van der Walt & D.B. Scott

H.J. Phaff and M.W. Miller

Diagnosis of the genus

Cells are long-ovoidal to cylindrical. In liquid media they occur in branched chains; on solid media they are mainly singles or pairs. Vegetative reproduction is by budding at the poles or on broad shoulders. In liquid media a sediment is formed.

Ascospores are ovoidal to cylindrical, one to four per ascus. No conjugation of cells prior to sporulation. Upon germination an exosporium is evident.

Fermentation of sugars is weak. Growth occurs only between 30 and 40°C. Amino acids, B-vitamins, and an increased level of CO_2 in the gas atmosphere are required for growth. The cells are short-lived at room temperature or above. Diazonium blue B reaction is negative.

Type species

Cyniclomyces guttulatus (Robin) van der Walt & D.B. Scott

Species accepted

1. *Cyniclomyces guttulatus* (Robin) van der Walt & D.B. Scott (1971)

Systematic discussion of the species

25.1. *Cyniclomyces guttulatus* (Robin) van der Walt & D.B. Scott (1971b)

Synonyms:
Cryptococcus guttulatus Robin (1853)
Saccharomyces guttulatus (Robin) Winter (1884)
Saccharomycopsis guttulatus (Robin) Schiönning (1903)
Atelosaccharomyces guttulatus (Robin) de Beurmann & Gougerot (1909)

Growth in liquid medium: (1% w/v yeast autolyzate, 1% Proteose peptone, 2% glucose, pH 4.5). After one day at 37°C, cells are long-ovoidal to cylindrical (Fig. 46). Budding occurs polar and at the shoulders of the cells, resulting in branched pseudomycelial formations. Often, the original parent cell appears dead by the time the chain is formed, as judged by complete granulation of its cytoplasm. After 48 h the range of cell dimensions is (4.4–6.1)×(14–21) µm. The cells have a tendency to grow on the glass wall of a tube, about 1 cm below the surface of the liquid. In older cultures the cells form a loose, often granular sediment. No ring or pellicle is formed even after an extended period of incubation.

Growth on solid medium: When a plate of yeast autolyzate–Proteose-peptone–glucose agar (pH 4.5) is inoculated with cells of *C. guttulatus,* smooth, semiglossy colonies appear after 24 h, provided the plates are incubated in an atmosphere high in CO_2 content (optimal concentration 15%). Cells are ovoidal to cylindrical, (4.7–7.9)×(8.8–19.5) µm, mainly single or in pairs.

Formation of ascospores: No conjugation immediately preceding ascospore formation; the vegetative cells are presumed to be diploid. Asci contain one to four (rarely more) ovoidal to cylindrical ascospores, (3.6–4.8)×(7.0–10) µm (Fig. 47), which are lying free in the ascus. This arrangement is quite different from the tightly fitting spores in asci of species of *Saccharomyces*. Asci rarely deliquesce at maturity. Ascospores germinate directly or after conjugation with another spore. Germination tubes

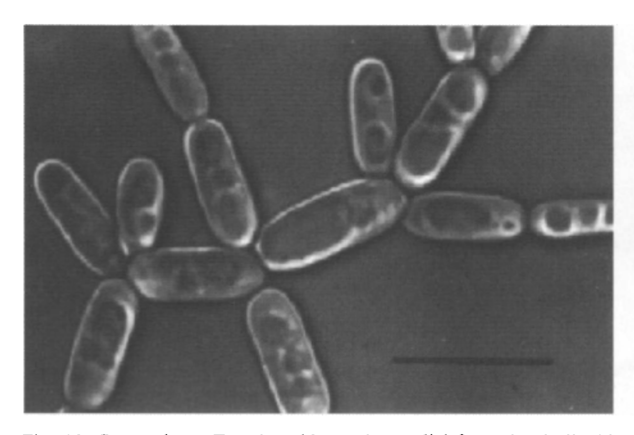


Fig. 46. *C. guttulatus.* Two-day old pseudomycelial formation in liquid medium. Bar = 20 µm.

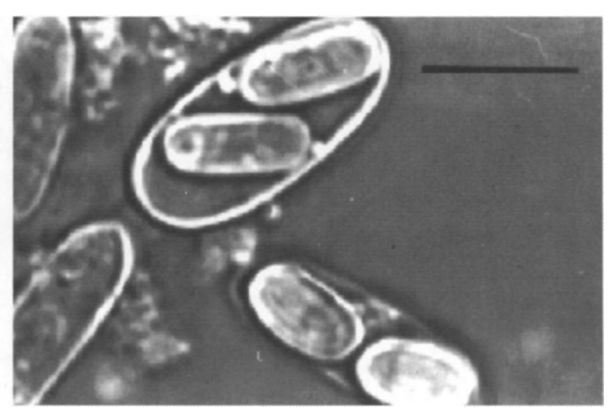


Fig. 47. *C. guttulatus.* Two-spored asci after 7 days at 18°C on sporulation medium. Bar = 12 µm.

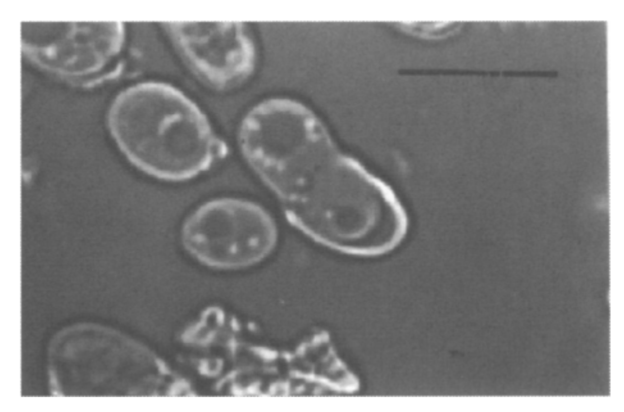


Fig. 48. *C. guttulatus.* Germination of a single ascospore. The exosporium is being shed at the base of the spore. Bar = 16 µm.

are formed with simultaneous shedding of an outer spore coat (exosporium) (Fig. 48). Germination is followed by formation of buds. Ascospores are formed only at temperatures lower than the growth temperature, best at about 18°C, where vegetative growth is not possible. Agar slants of the following composition are a suitable sporulation medium: 0.3% yeast autolyzate, 0.3% malt extract, 0.15% Bacto-peptone, 1% glucose. The slants are inoculated with about 1 ml sedimented cell material grown in a liquid medium. The slants are incubated in a horizontal position for 5–7 days at 18°C.

Fermentation:

Glucose	w	Raffinose	w,s
Galactose	–	Trehalose	–
Sucrose	w	Melibiose	–
Maltose	–	Inulin	–
Lactose	–		

Assimilation:

Assimilation tests were carried out in Yeast nitrogen base (w/o amino acids and w/o ammonium sulfate) plus 0.1% yeast extract and 0.5% Proteose–peptone in screw-capped test tubes flushed with CO_2 before autoclaving. Control tubes without glucose showed no growth. The tubes were incubated at 37°C on a Rollordrum.

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	w/–	Salicin	–
Inulin	l/w	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	w
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	n
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Cycloheximide 10 µg/ml	–
5-Keto-D-gluconate	–	Growth at 37°C	+
5% NaCl/5% glucose	–		

Temperature range for growth is 30–37°C in the presence of CO_2. Vitamins required for growth are nicotinic acid, pantothenic acid, thiamin, and inositol (Shifrine and Phaff 1959).

Co-Q: 6, Strain UCD-FST 73-90 (Yamada and Phaff, unpublished results).

Mol% G + C: 34.5, Strain UCD-FST 73-90 (BD: Mendonça-Hagler and Phaff 1975).

Origin of the strain studied: UCD-FST 92-2, a newly isolated strain from a domestic rabbit.

Type strain: Phaff (1970b) designated a newly isolated strain from a domestic rabbit as the type (CBS 5913), since no designated type strain existed at that time. Phaff and Miller (1984a) also designated CBS 5913 as the type strain. However, the 1987 CBS catalogue listed CBS 8137, isolated by D. Yarrow from a rabbit, as the only strain available because the original type was no longer viable.

Comments on the genus

The long history of the genus *Cyniclomyces* has been reviewed by Phaff (1970b) and by van der Walt and Scott (1971b). Buecher and Phaff (1970) studied the requirements of various gasses for growth in the atmosphere. *C. guttulatus* grew exponentially at a maximal growth rate under a continuous gas phase of 15% CO_2, 2% O_2 in nitrogen. The growth rates were almost the same when the oxygen concentration was varied between 0.25 and 20%, but in the absence of oxygen, growth was poor. Buecher and Phaff (1972) also isolated and described an atypical dimorphic, filamentous strain of *C. guttulatus* from a wild jack rabbit. The colonies of this strain were irregular, rough, and dull and the vegetative growth consisted of septate, branched hyphae, radiating from the central origin of growth. New filaments originated by fragmentation of older aggregates. The atypical strain was not CO_2 dependent at 37°C but did require oxygen. At 30°C, the lower limit for growth, a morphogenetic change took place from the filamentous form to the yeast form, which was stimulated by anaerobic conditions. The budding form of the filamentous strain was relatively stable in culture; at either 30 or 37°C it was CO_2 dependent. Spontaneous reversions from the budding to the filamentous form took place sporadically. Unfortunately this strain has not been preserved.

One of the problems of working with *C. guttulatus* is the high death rate of the cells. The low pH required for the isolation of this yeast causes early granulation and death of the cells. If sufficient CO_2 is not present and the inoculum contains low cell numbers, the cells may die before they can grow out and generate their own CO_2 in liquid media. This species is therefore difficult to maintain in culture. A lyophilized preparation of a young sporulating culture is recommended for extended storage. Sporulating slants

may be stored in a refrigerator for 6–12 months. Vegetative growth on slants of 1% yeast autolyzate, 1% Proteose peptone–2% glucose, pH 5.0 can be stored for about one month in a refrigerator. Reinoculation of preserved material into liquid growth medium at 37°C in a container with elevated CO_2 atmosphere results in good growth after 4–7 days. More recently, Zierdt et al. (1988) recommended a very complex new medium, "Fastidious Microorganism Medium" with or without agar. They claimed that elevated CO_2 levels were not required for growth on agar medium. We prepared the new FMM medium and found that after addition of 1% glucose, growth at 37°C in air was poor and slow. In the presence of 10% CO_2 growth was moderate to good, but somewhat less vigorous than on our Yeast autolyzate–Proteose peptone-glucose medium. No growth occurred on either medium in the absence of glucose. Because the FMM medium is costly to prepare, we recommend our medium described in this chapter. Zierdt et al. (1988) also produced some excellent ultrastructural TEM photographs of vegetative cells and asci of *C. guttulatus*.

Because *C. guttulatus* is short-lived and thus easily lost in collections, a brief description is given of a reliable isolation procedure.

Isolation principle: The yeast is most easily isolated from stomach contents of a rabbit, but because fecal material is more readily available, the procedure described below is based on fecal pellets. The yeast requires a balanced mixture of amino acids and vitamins (as found in yeast autolyzate and Proteose peptone), grows between 30 and 40°C, and, for growth on solid media, it requires an elevated concentration of CO_2 in the atmosphere. Additionally, it is very tolerant to low pH values of the medium. Use is made of these properties to isolate pure cultures.

Method of isolation: Samples of fecal pellets of rabbits, preferably samples of soft feces (the latter normally have a higher yeast content) are inspected microscopically for the presence of the large *C. guttulatus* cells by suspending small samples in a drop of water on a microscope slide. Material in which the yeast is present in significant numbers gives the best results. The isolation medium contains 1% powdered yeast autolyzate, 1% Proteose peptone (Difco), 2% glucose and the pH is adjusted to 2.8–3.0 with 1 N HCl. Fifty-ml portions of this medium in 125-ml Erlenmeyer flasks are sterilized in flowing steam for 10 min.

1. Add one or two rabbit pellets to a flask and incubate for 2–3 days at 37°C. A whitish, granular sediment of yeast forms at the bottom, which should consist of clusters and chains of the characteristic, large cells of *C. guttulatus*.

2. If the sediment appears satisfactory, transfer 1 ml of the sediment with a sterile pipet to a second flask of the same medium and incubate at 37°C.

3. After growth has developed, transfer two drops of the sediment to an agar plate (1% yeast autolyzate, 1% Proteose peptone, 2% glucose, pH 5.0) and spread with a loop. The plate is incubated in a small desiccator with a little water in the bottom to raise the humidity. Flush the desiccator with CO_2 from a cylinder or place a short piece of lighted candle in the desiccator and put the lid in place. After a few minutes, when the CO_2 level has increased, the candle extinguishes and the desiccator is placed at 37°C for 3 days.

4. An isolated colony is then inspected for purity. If bacteria are not eliminated during the previous steps, they would develop on the rich medium at pH 5.0. If the colony is pure, it is transferred to a slant and to a liquid medium of the same composition as used in step 3 and incubated as described above.

Note: Growth in liquid medium is always in the form of branched chains of cells, whereas on solid medium the cells occur more typically in short chains, pairs, or singly.

26. *Debaryomyces* Lodder & Kreger-van Rij Nom. Cons.

T. Nakase, M. Suzuki, H.J. Phaff and C.P. Kurtzman

Diagnosis of the genus

Vegetative reproduction is by multilateral budding. Pseudomycelium is absent, primitive, or occasionally well developed.

Heterogamous conjugation between a cell and its bud generally precedes ascus formation. Isogamous conjugation also occurs. Ascospores are spheroidal, globose, ovoidal or lenticular and have smooth or warty walls (verrucose to colliculate) that may also have an equatorial ledge or spiral ridges. Ascospores are not liberated from the ascus except for three species, whose asci lyse. One to two spores are usually formed per ascus; in some species up to four spores are present in the ascus.

Fermentation is absent, weak or occasionally vigorous. Nitrate is not assimilated. The major ubiquinone is Q-9. Diazonium blue B reaction is negative.

Type species

Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij

Species accepted

1. *Debaryomyces carsonii* (Phaff & Knapp) Y. Yamada, Maeda, Banno & van der Walt (1992)
2. *Debaryomyces castellii* Capriotti (1958)
3. *Debaryomyces coudertii* Saëz (1960)
4. *Debaryomyces etchellsii* (Kreger-van Rij) Maeda, Y. Yamada, Banno & van der Walt (1992)
5. *Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij (1952)
   a. *Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij var. *hansenii* (1985)
   b. *Debaryomyces hansenii* var. *fabryi* (Ota) Nakase & M. Suzuki (1985)
6. *Debaryomyces maramus* di Menna (1954)
7. *Debaryomyces melissophilus* (van der Walt & van der Klift) Kurtzman & Kreger-van Rij (1976)
8. *Debaryomyces nepalensis* S. Goto & Sugiyama (1968)
9. *Debaryomyces occidentalis* (Klöcker) Kurtzman & Robnett (1991)
   a. *Debaryomyces occidentalis* (Klöcker) Kurtzman & Robnett var. *occidentalis* (1991)
   b. *Debaryomyces occidentalis* var. *persoonii* (van der Walt) Kurtzman & Robnett (1991)
10. *Debaryomyces polymorphus* (Klöcker) Price & Phaff (1979)
11. *Debaryomyces pseudopolymorphus* (C. Ramírez & Boidin) Price & Phaff (1979)
12. *Debaryomyces robertsiae* (van der Walt) Kurtzman & Robnett (1994)
13. *Debaryomyces udenii* van der Walt, M.Th. Smith & Y. Yamada (1989)
14. *Debaryomyces vanrijiae* (van der Walt & Tscheuschner) Abadie, Pignal & J.L. Jacob (1963)
   a. *Debaryomyces vanrijiae* (van der Walt & Tscheuschner) Abadie, Pignal & J.L. Jacob var. *vanrijiae* (1984)
   b. *Debaryomyces vanrijiae* var. *yarrowii* (Santa María & García Aser) Kreger-van Rij (1984)
15. *Debaryomyces yamadae* (van der Walt & E. Johannsen) van der Walt, M.Th. Smith & Y. Yamada (1989)

Key to species

See Table 20.

1.	a	Sucrose assimilated → 2		
	b	Sucrose not assimilated	*D. coudertii*:	p. 160
2(1).	a	D-Xylose assimilated → 3		
	b	D-Xylose not assimilated → 4		
3(2).	a	Growth in vitamin-free medium → 5		
	b	Absence of growth in vitamin-free medium → 8		
4(2).	a	Erythritol assimilated	*D. melissophilus*:	p. 164
	b	Erythritol not assimilated	*D. occidentalis* var. *persoonii*:	p. 165
5(3).	a	Trehalose fermented	*D. robertsiae*:	p. 168
	b	Trehalose not fermented → 6		
6(5).	a	Fermentation of glucose and sucrose	*D. polymorphus*:	p. 166
	b	Fermentation of glucose and sucrose is very weak or absent → 7		
7(6).	a	Melibiose assimilated	*D. vanrijiae* var. *vanrijiae*:	p. 170
	b	Melibiose not assimilated	*D. vanrijiae* var. *yarrowii*:	p. 170

8(3). a D-Gluconate assimilated → 9
b D-Gluconate not assimilated → 10
9(8). a Raffinose assimilated → 11
b Raffinose not assimilated .... *D. yamadae*: p. 171
10(8). a Glucose fermented .... *D. etchellsii*: p. 160
b Glucose not fermented .... *D. carsonii*: p. 158
11(9). a Erythritol assimilated → 12
b Erythritol not assimilated → 15
12(11). a Fermentation of glucose and sucrose → 13
b Fermentation of glucose and sucrose is very weak or absent → 14
13(12). a Lactose assimilated .... *D. pseudopolymorphus*: p. 167
b Lactose not assimilated .... *D. udenii*: p. 169
14(12). a Ascospores spheroidal, one to two per ascus .... *D. hansenii* var. *hansenii*: p. 161
D. hansenii var. *fabryi*: p. 162
D. nepalensis: p. 164
b Ascospores ovoidal, one to four per ascus .... *D. maramus*: p. 163
15(11). a Growth in 10% NaCl/5percnt; glucose → 16
b Absence of growth in 10% NaCl/5% glucose .... *D. occidentalis* var. *occidentalis*: p. 165
16(15). a Fermentation of glucose and sucrose .... *D. castellii*: p. 159
b Fermentation of glucose and sucrose is very weak or absent .... *D. hansenii* var. *hansenii*: p. 161
D. hansenii var. *fabryi*: p. 162

Table 20
Key characters of species in the genus *Debaryomyces*

Species	Fermentation[a]			Assimilation							10% NaCl/ 5% G	Vit	Ascospores		
	G	Su	Tr	Su	La	Me	Ra	Xy	Gl	Er			Shape	Num[b]	Lib[c]
Debaryomyces carsonii	–	–	–	+	–	v	v	+	–	–	+	–	spheroidal	1–4	+
D. castellii	+	+	–	+	+	+	+	+	+	–	+	–	spheroidal	1–3 (1)	–
D. coudertii	–	–	–	–	–	–	–	+	+	+	+	–	spheroidal	1	–
D. etchellsii	×	w/–	–	+	–	–	–	+	–	–	+	–	spheroidal	1-4	+
D. hansenii var. *hansenii*	w/–	w/–	w/–	+	v	v	+	+	×	v	+	–	spheroidal	1–2 (1)	–
D. hansenii var. *fabryi*	w/–	w/–	w/–	+	v	v	+	+	×	v	+	–	spheroidal	1–2 (1)	–
D. maramus	w/–	–	–	+	v	v	s	+	+	+	+	–	ovoidal	1–4 (2)	–
D. melissophilus	–	–	–	+	–	–	–	–	+	+	+	–	spheroidal	1–4	–
D. nepalensis	w/–	w/–	w/–	+	v	+	+	+	+	+	+	–	spheroidal	1	–
D. occidentalis var. *occidentalis*	+	+	–	+	v	v	+	+	+	–	–	–	spheroidal with equatorial ledge	1–2 (1)	–
D. occidentalis var. *persoonii*	+	+	–	+	+	v	+	–	+	–	–	–	spheroidal with equatorial ledge	1–2 (1)	–
D. polymorphus	s	s	–	+	v	v	+	+	+	+	+	+	spheroidal	1–2 (1)	–
D. pseudopolymorphus	+	s	–	+	+	+	+	+	+	+	+	–	spheroidal	1–4	–
D. robertsiae	+	+	+	+	–	–	+	+	+	+	+	+	lenticular	1–4	–
D. udenii	ws	w/–	w/–	+	–	+	+	+	+	+	+	–	spheroidal	1–4	+
D. vanrijiae var. *vanrijiae*	w/–	w/–	–	+	–	+	+	+	+	+	+	+	spheroidal	1–4	–
D. vanrijiae var. *yarrowii*	w/–	w/–	–	+	–	–	+	+	+	v	+	+	spheroidal	1–4	–
D. yamadae	s	–	–	+	+	–	–	+	w	–	v	–	spheroidal	1–4	–

[a] Abbreviations: G, glucose; Su, sucrose; Tr, trehalose; La, lactose; Me, melibiose; Ra, raffinose; Xy, D-xylose; Gl, gluconate; Er, erythritol; 10% NaCl/5% G, growth in 10% NaCl/5% glucose; Vit, growth in vitamin-free medium; +, positive; s, positive but slow; ×, positive or weak; w, weak; ws, weak and slow; w/–, weak or negative; v, variable; –, negative.
[b] Numbers of ascospores per ascus; numbers in parentheses refer to the number of ascospores most frequently observed.
[c] Ascospores liberated by lysis of asci.

Systematic discussion of the species

26.1. *Debaryomyces carsonii* (Phaff & Knapp) Y. Yamada, Maeda, Banno & van der Walt (1992d)

Synonyms:
Pichia carsonii Phaff & Knapp (1956)
Torulaspora carsonii (Phaff & Knapp) van der Walt & E. Johannsen (1975a)
Debaryomyces vini Zimmermann (1938) nom. nud.
Pichia vini (Zimmermann) Phaff (1956) nom. nud.
Pichia vini (Zimmermann) Phaff var. *melibiosi* Santa María (1963a) nom. nud.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to elongate (1.4–4.2)×(2.0–6.0) µm, and occur singly, in pairs and in chains. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin to moderately heavy pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant but sparingly branched pseudohyphae. True hyphae are not produced. Aerobic growth is tannish-white, dull to faintly glistening, butyrous and with an entire or occasionally notched margin.

Formation of ascospores: Asci may show conjugation between a cell and its bud or may be unconjugated. One to four smooth spheroidal spores form in each ascus, and asci may be persistent or deliquescent. Single-spore isolates from four-spored asci are sporogenous, thus indicating the species to be homothallic.

Ascospores were observed on YM agar after 5–10 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	+/w
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 2285, CBS 810 (Yamada et al. 1973a).

Mol% G+C: 36.8, 37.3, IFO 795, CBS 5254 (T_m: Nakase and Komagata 1970b); 38.7, 38.9, CBS 810, CBS 5254 (BD: Price et al. 1978); 39.6-39.7, CBS 2285, CBS 810, CBS 5254 (BD: Kurtzman and Smiley 1979).

Origin of the strains studied: Wine, Germany and South Africa (2); *Quercus kelloggii* U.S.A. (2); soil, Japan (1); 'alpechin', Spain (1); *Aulacigaster* sp., U.S.A. (1).

Type strain: CBS 2285 (NRRL YB-4275), from *Quercus kelloggii.*

Comments: *Pichia vini* was first isolated by Zimmermann (1938) from spoiled wine and described as *Debaryomyces vini.* Later, Phaff (1956) transferred this species when he emended the genus *Pichia.* Several additional strains were isolated and appeared identical to the original strain except for the presence of deliquescent asci (Kreger-van Rij 1970c). Santa María (1963a) obtained two similar cultures from 'alpechin' but described them as *P. vini* var. *melibiosi* because they assimilated melibiose and raffinose.

Pichia carsonii described by Phaff and Knapp (1956), appeared identical with *P. vini* except for its hat-shaped ascospores. Kreger-van Rij (1970c) showed by transmission electron microscopy that the rather small spores of *P. carsonii* were actually spheroidal and practically smooth. Consequently, she considered this species a synonym of *P. vini.* Van der Walt and Johannsen (1975a) pointed out that Zimmermann (1938) failed to provide a Latin diagnosis for *Debaryomyces vini* and that *P. carsonii* was the legitimate name. However, they transferred the species to *Torulaspora* as *T. carsonii* when they revised that genus.

Kurtzman and Smiley (1979) demonstrated by scanning electron microscopy that the ascospores from all extant strains of *Pichia carsonii* and its synonyms were spheroidal and essentially smooth. Furthermore, they showed by DNA reassociation that the type strains of *Pichia vini, P. vini* var. *melibiosi* and *P. carsonii* were highly related and represented the same species. They rejected the transfer of *Pichia carsonii* to *Torulaspora,* pointing out that the originally described species of that genus have roughened ascospore walls and coenzyme Q-6 in the electron transport system, whereas *P. carsonii* has smooth-walled ascospores and forms coenzyme Q-9.

Yamada et al. (1992b) compared partial 18S and 26S rRNA sequences from *P. carsonii* and *P. etchellsii* and noted their similarity to species of *Debaryomyces.* From this analysis, Yamada et al. (1992d) transferred *P. carsonii* and *P. etchellsii* to *Debaryomyces.*

26.2. *Debaryomyces castellii* Capriotti (1958e)

Synonyms:

Zymodebaryomyces castellii (Capriotti) Novák & Zsolt (1961)
Pichia castellii (Capriotti) Campbell (1973)
Torulaspora castellii (Capriotti) van der Walt & E. Johannsen (1975a)
Debaryozyma castellii (Capriotti) van der Walt & E. Johannsen (1978)

Growth in YM broth: After 3 days at 25°C, the cells are spheroidal to short-ovoidal, (3.8–7.5)×(5–8.5) μm, and single, in pairs or in groups. A sediment and a thin, dull, creeping pellicle are formed.

Growth on YM agar: After one month at 17°C, the streak culture is yellowish-white, dull or glistening, smooth, with an occasionally sinuous margin.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed. Short, cylindrical cells may be present.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. The spores are spheroidal with a warty wall. The warts are not always distinctly visible with the light microscope. One to three, but generally one spore per ascus (Fig. 49).

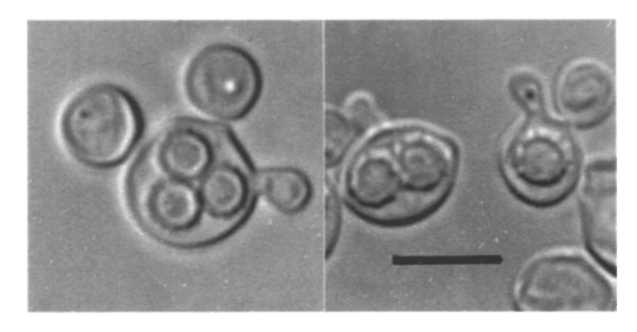


Fig. 49. *D. castellii*, CBS 2923. Asci with ascospores, after one month at 20°C on V8 agar. Bar = 5 µm.

Ascospores were observed on V8, Gorodkowa, and acetate agars after 1–2 weeks at 20°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	w		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	s
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	s
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+/w	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	+
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Growth at 37°C	–

Co-Q: 9 (minor components 8 and 10), CBS 2923 (Billon-Grand 1987).
Mol% G+C: 37.1, CBS 2923 (BD: Price et al. 1978).

Origin of the strain studied: Soil in Sweden (CBS 2923).

Type strain: CBS 2923, isolated by Capriotti.

26.3. *Debaryomyces coudertii* Saëz (1960)

Synonyms:
Pichia coudertii (Saëz) Campbell (1973)
Torulaspora coudertii (Saëz) van der Walt & E. Johannsen (1975a)
Debaryozyma coudertii (Saëz) van der Walt & E. Johannsen (1978)

Growth in YM broth: After 3 days at 25°C, the cells are spheroidal to short-ovoidal, (3.5–5.1)×(4–5.8) µm, and single or in pairs. A thin sediment is formed.

Growth on YM agar: After one month at 17°C, the streak culture is yellowish-white, dull to shiny, soft, slightly raised in the middle and smooth.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. The spores are spheroidal with a warty wall; one spore is formed per ascus. The presence of many spores gives the culture a brown appearance.

Ascospores were observed on V8 agar after 1–2 weeks at room temperature.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	s
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	s	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	+
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Growth at 37°C	–

Co-Q: 9 (minor component 10), CBS 5167 (Billon-Grand 1987).
Mol% G+C: 37.4, CBS 5167 (BD: Price et al. 1978).

Origin of the strain studied: Feces of *Aptenodytes patagonica*, the emperor penguin (CBS 5167).

Type strain: CBS 5167, isolated by Saëz (1960).

Comments: In the description by Kreger-van Rij (1970a, 1984d), *D. coudertii* was reported not to assimilate L-rhamnose, but in the present study L-rhamnose was assimilated. Assimilation of this sugar was also reported by Barnett et al. (1983, 1990).

26.4. *Debaryomyces etchellsii* (Kreger-van Rij) Maeda, Y. Yamada, Banno & van der Walt (Yamada et al. 1992d)

Synonyms:
Pichia etchellsii Kreger-van Rij (1964a)
Torulaspora etchellsii (Kreger-van Rij) van der Walt & E. Johannsen (1975a)
Kloeckera faecalis Batista & Silveira (1959a)
Pichia faecalis (Batista & Silveira) Aciole de Queiroz (1970)

Growth on 5% malt extract agar: After 3 days at

25°C, the cells are ovoidal to ellipsoidal (1.8–5.4)×(2.0–8.3) μm, and occur singly or in pairs. Growth is white, dull-glistening and butyrous.

Growth on the surface of assimilation media: Occasional rings are noted, but pellicles do not form.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows moderately well-branched pseudohyphae with few blastospores. True hyphae are not present. Aerobic growth on morphology agar is white, dull-glistening and butyrous with a finely striated margin.

Formation of ascospores: Asci show conjugation between a cell and its bud, or infrequently, conjugation between independent cells. One to four smooth, spheroidal ascospores are formed per ascus, and the asci eventually become deliquescent. Single-spore isolates gave sporogenous colonies, indicating the species to be homothallic.

Ascospores were observed on YM agar after 8–10 days at 25°C.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	w/–	Trehalose	–
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	w/s
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 2011 (Yamada et al. 1973a).

Mol% G+C: 38.5, 40.6, CBS 2011 (T_m: Nakase and Komagata 1970b; BD: Price et al. 1978).

Origin of the strains studied: Fermenting cucumber brines, U.S.A. (2); human feces (1).

Type strain: CBS 2011 (NRRL Y-7121), one of the strains from cucumber brines.

Comments: *Debaryomyces etchellsii* is phenotypically similar to *D. carsonii*, differing from the former only by its ability to ferment and by its failure to assimilate starch. However, Kurtzman (unpublished) found only 2% DNA base sequence complementarity between type strains of the two and considered them separate species.

As with *D. carsonii, D. etchellsii* has Q-9 ubiquinone in the electron transport system (Yamada et al. 1973a), and its ascospores are spheroidal and smooth when viewed under the scanning electron microscope (Kurtzman and Smiley 1974). For these reasons, its placement in the genus *Torulaspora* by van der Walt and Johannsen (1975a) was rejected. On the basis of rRNA comparisons, Yamada et al. (1992d) transferred *Pichia carsonii* and *P. etchellsii* to *Debaryomyces*.

26.5. *Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij (1952)

This species has two varieties:

Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij var. *hansenii* (1985)

Anamorph: *Candida famata* (Harrison) S.A. Meyer & Yarrow var. *famata*

Synonyms:

Saccharomyces hansenii Zopf (1889)
Pichia hansenii (Zopf) Campbell (1973)
Torulaspora hansenii (Zopf) van der Walt & E. Johannsen (1975a)
Debaryozyma hansenii (Zopf) van der Walt & E. Johannsen (1978)
Debaryomyces tyrocola Konokotina (1913)
Debaryomyces tyrocola Konokotina var. *hansenii* (Zopf) Dekker (Stelling-Dekker 1931)
Debaryomyces membranaefaciens Naganishi (1917)
Debaryomyces kloeckeri Guilliermond & Péju (1919)
Saccharomyces kloeckeri (Guilliermond & Péju) Brumpt (1927)
Debaryomyces matruchoti Grigoraki & Péju (1921)
Atelosaccharomyces hudeloi de Beurmann & Gougerot (1910)
Debaryomyces hudeloi (de Beurmann & Gougerot) da Fonseca (1922)
Debaryomyces kloeckeri Guilliermond & Péju var. *hudeloi* (de Beurmann & Gougerot) Dekker (Stelling-Dekker 1931)
Torula candida Saito (1922)
Torulopsis candida (Saito) Lodder (1934)
Cryptococcus candidus (Saito) Skinner (1950)
Debaryomyces hildegaardi Ota (1923)
?*Debaryomyces laedegaardi* Ota (1923)
?*Debaryomyces leopoldi* Ota (1923)
?*Debaryomyces lundsgaardi* Ota (1923)
Debaryomyces gruetzii Ota (1924b)
?*Debaryomyces tremoniensis* Ota (1924b)
?*Debaryomyces fabryi* Ota var. *tremoniensis* (Ota) Dodge (1935)
?*Debaryomyces emphysematosus* Ota (1924b)
Mycotorula famata Harrison (1928)
Geotrichum famatum (Harrison) Dodge (1935)
Torulopsis famata (Harrison) Lodder & Kreger-van Rij (1952)
Candida famata (Harrison) Novák & Zsolt (1961) nom. inval.
Candida famata (Harrison) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Debaryomyces matruchoti Grigoraki & Péju var. *cesarii* Dekker (Stelling-Dekker 1931)
Debaryomyces guilliermondii Dekker (Stelling-Dekker 1931)
Debaryomyces guilliermondii Dekker var. *nova-zeelandicus* Lodder (1932)
Debaryomyces membranaefaciens Naganishi var. *hollandicus* Lodder (1932)
Debaryomyces kloeckeri Guilliermond & Péju var. *major* Lodder (1932)

Debaryomyces sake Saito & Oda (1932)
?*Cryptococcus minor* Pollacci & Nannizzi (Nannizzi 1934)
?*Torulopsis minor* (Pollacci & Nannizzi) Lodder (1934)
?*Parendomyces minor* (Pollacci & Nannizzi) Dodge (1935)
?*Rhodotorula minor* (Pollacci & Nannizzi) Krasil'nikov (1954a)
Debaryomyces miso Mogi (1938a)
Debaryomyces nicotianae Giovannozzi (1939)
Debaryomyces nicotianae Giovannozzi var. *minor* Giovannozzi (1939)
Debaryomyces marylandii Giovannozzi (1941)
?*Candida anomala* C. Ramírez Gómez (1957)
Paratorulopsis banheggii Galgóczy & Novák (1962)
Torulopsis westerdijkii Novák & Vitéz (1964)
?*Candida periphelosum* Nagasawa, Ono, Kudo & Harada (1975) nom. nud.
Torulopsis armenti Kocková-Kratochvílová, Sláviková & Beránek (1977a)

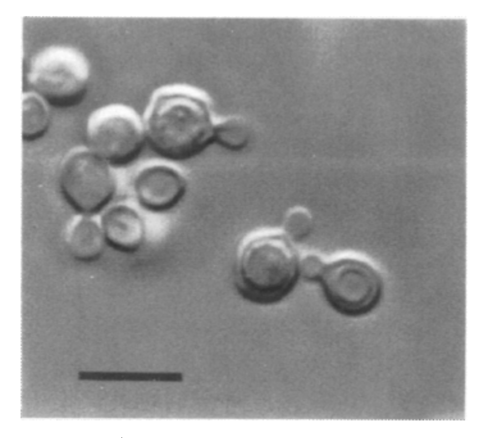


Fig. 50. *D. hansenii* var. *hansenii*, JCM 2093. Asci with one ascospore each, after one month on V8 agar at 20°C. Bar = 5 µm.

Debaryomyces hansenii var. *fabryi* (Ota) Nakase & M. Suzuki (1985b)

Anamorph: *Candida famata* (Harrison) S.A. Meyer & Yarrow var. *flareri* (Ciferri & Redaelli) Nakase & M. Suzuki

Synonyms:
Blastodendrion flareri Ciferri & Redaelli (1935)
Parendomyces flareri (Ciferri & Redaelli) Dodge & Moore (1936)
Candida flareri (Ciferri & Redaelli) Langeron & Guerra (1938)
Candida famata (Harrison) S.A. Meyer & Yarrow var. *flareri* (Ciferri & Redaelli) Nakase & M. Suzuki (1985b)
Debaryomyces fabryi Ota (1924b)
Eutorulopsis subglobosa Zach (Wolfram and Zach 1934b)
Debaryomyces matruchoti Grigoraki & Péju var. *subglobosus* Zach (Lodder and Kreger-van Rij 1952)
Debaryomyces subglobosus (Zach) Lodder & Kreger-van Rij (1952)
Debaryomyces fukuyamaensis Naganishi (1941b)
?*Debaryomyces orientalis* Naganishi (1941a)
Pichia adzetii F.H. Jacob (1969b)

Growth in YM broth: After 3 days at 25°C, the cells are spheroidal to short-ovoidal, (2–7.2)×(2.2–8.6) µm, and single, in pairs or in short chains. A sediment, a ring and, in some strains, a smooth or wrinkled, dry pellicle or islets are formed.

Growth on YM agar: After one month at 17°C, the streak culture is grayish-white to yellowish, soft, shiny or dull, smooth or partly or entirely striped or wrinkled.

Dalmau plate culture on corn meal agar: Pseudomycelium is usually lacking, but occasionally a primitive, or even a well-developed pseudomycelium is formed.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation; conjugation between separate cells may also occur. The spores are spheroidal with a warty wall. The wartiness is not always distinct under the light microscope. With the scanning electron microscope, warts appear as small blunt protuberances or as small ridges (Kurtzman et al. 1975). In two strains, a unique wale-type of surface ornamentation of ascospores was observed by Banno and Mikata (1985). Usually one, seldom two spores are formed per ascus (Fig. 50). The presence of many spores gives the culture a brown color.

Ascospores were observed on V8, Gorodkowa, acetate and YM agars after 1–2 weeks at or below 20°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	w/–	Raffinose	w/–
Sucrose	w/–	Trehalose	w/–
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	v
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+/w
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	+/w
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+/w
Inulin	v	D-Gluconate	+/w
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+/w	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	v
L-Rhamnose	v	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	v
5-Keto-D-gluconate	v	Starch formation	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+	Max. growth temp. (°C)	31–35

Co-Q: 9 (minor component 8) (Nakase and Suzuki 1985a).

Mol% G + C: 38.2–38.6, type strain and 5 additional strains (BD: Price et al. 1978); 36.3–37.8 mol%, type strain and 33 additional strains (T_m: Nakase and Suzuki 1985a), for the variety *hansenii;* 37.3 mol%, CBS 792 (Price et al. 1978), 36.4–36.8 mol%, type strain and 5 additional strains (T_m: Nakase and Suzuki 1985a), for the variety *fabryi.*

Supplemental description of *D. hansenii* var. *fabryi*: The variety *fabryi* can be physiologically distinguished from the variety *hansenii* only by maximum growth temperature. The maximum growth temperature of the variety *fabryi* is 36–39°C, whereas that of the variety *hansenii* is 31–35°C. Growth at 37°C by the variety *fabryi* may be variable.

Origin of the strains belonging to the variety *hansenii*: Cheese, CBS 766 and three strains; sausages, CBS 773 and CBS 791; sake moto, CBS 2333 = JCM 2111; edomiso, CBS 2331 = JCM 2162; rennet, CBS 772; tobacco, CBS 811 and 2 other strains; psoriasis, CBS 161; infected nails, CBS 790; infected hand, CBS 5704; patient with angina, CBS 770; wound, CBS 788; salmon (shake in Japanese), JCM 2213; unknown, NRRL Y-7393 and 8 other strains.

Type strain: CBS 767, a strain with the name *Saccharomyces hansenii* received in the Delft CBS collection from the Carlsberg Laboratory.

Origin of the strains belonging to the variety *fabryi*: Interdigital mycotic lesion, CBS 789 = JCM 2104; tanning liquor, CBS 6066, type strain of *Pichia adzetii;* infected nail, CBS 792, type strain of *D. subglobosus;* rice vinegar mash, JCM 2207 = IFO 0037; skin lesion, CBS 1796; spoiled sake, JCM 1527 = IFO 0059.

Type strain: CBS 789.

Comments: Price et al. (1978) found that *D. subglobosus* (CBS 792) and *D. nepalensis* (CBS 5921) showed 39.7% and 15.4% relative DNA relatedness to the type strain of *D. hansenii* (CBS 767), respectively. Nakase and Suzuki (1985a,b) further investigated strains of *D. hansenii,* its anamorphs and *D. nepalensis* chemotaxonomically, and divided them into two species and two varieties based on DNA–DNA hybridization, i.e., *D. hansenii* var. *hansenii* (anamorph *Candida famata* var. *famata*), *D. hansenii* var. *fabryi* (anamorph *Candida famata* var. *flareri*) and *D. nepalensis* (anamorph *Candida naganishii*). Also, they showed *D. subglobosus* to be a synonym of *D. hansenii* var. *fabryi*. *Debaryomyces hansenii* var. *hansenii* can be distinguished from the variety *fabryi* and *D. nepalensis* by its lower maximum growth temperature and the lack of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity. Almost all strains of *D. hansenii* var. *hansenii* can be clearly separated from the variety *fabryi* and *D. nepalensis* by the difference in maximum growth temperature. In the case of some strains, the separation of these taxa by enzyme patterns is more useful. The separation of *D. hansenii* var. *fabryi* from *D. nepalensis* based on maximum growth temperature is difficult because their ranges overlap. At present, these taxa can be distinguished from each other only by the different banding pattern of malate dehydrogenase (EC 1.1.1.37).

26.6. *Debaryomyces maramus* di Menna (1954a)

Synonyms:
Pichia marama (di Menna) Phaff (1956)
Zymodebaryomyces marama (di Menna) Novák & Zsolt (1961)

Growth in YM broth: After 3 days at 25°C, the cells are spheroidal to short-ovoidal, (2–4)×(3.4–9) μm, and single, in pairs or in chains. A sediment, a ring and, occasionally, a thin dull pellicle are formed.

Growth on YM agar: After one month at 17°C, the streak culture is yellowish-white, soft, shiny to dull, and smooth.

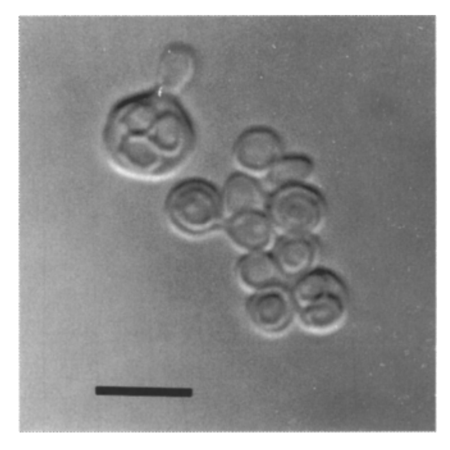


Fig. 51. *D. maramus,* CBS 1958. Asci with ascospores, after one month on YM agar at 20°C. Bar = 5 μm.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed. Short cylindrical cells may be present.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. The spores are ovoidal. In the scanning electron microscope, they have spiral ridges (Kurtzman et al. 1975). Under the light microscope they may appear to be smooth. One to four, usually two, spores are formed per ascus (Fig. 51). The presence of many spores gives the culture a brown color.

Ascospores were observed on V8 and YM agars after 1–2 weeks at or below 20°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	s	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	v	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	w/–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	+
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Growth at 37°C	–

Co-Q: 9 (minor components 8 and 10), CBS 1958 (Billon-Grand 1987; Yamada et al. 1976b).
Mol% G+C: 39.1, CBS 1958 (BD: Price et al. 1978).

Origin of the strains studied: Air in New Zealand, CBS 1958 = JCM 1528; cider, CBS 4264 = JCM 6179 = NRRL Y-7427; possible case of human disease, CBS 1794 = JCM 6178; soil in Iran, CBS 6905 = JCM 6277.

Type strain: CBS 1958, isolated by di Menna in New Zealand.

Comments: *D. maramus* is the only *Debaryomyces* species with spiral ridges on the spore wall.

26.7. *Debaryomyces melissophilus* (van der Walt & van der Klift) Kurtzman & Kreger-van Rij (1976)

Synonyms:
Pichia melissophila van der Walt & van der Klift (1972)
Torulopsis melissophila van der Walt & van der Klift (1972)
Torulaspora melissophila (van der Walt & van der Klift) van der Walt & E. Johannsen (1975a)
Debaryozyma melissophila (van der Walt & van der Klift) van der Walt & E. Johannsen (1980)

Growth in YM broth: After 3 days at 25°C, the cells are short-ovoidal or ovoidal, $(1.4–4.2)\times(2.4–5.6)$ µm, and single, in pairs or in short chains. A sediment is formed.

Growth on YM agar: After one month at 17°C, the streak culture is cream-colored, soft, smooth and dull to shiny.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed.

Formation of ascospores: Conjugation between a cell and its bud or between separate cells precedes ascus formation. The spores are spheroidal with a warty wall which is detectable by scanning electron microscopy and in ultrathin sections (Kurtzman and Kreger-van Rij 1976), but not under the light microscope. One to four spores are formed in the zygote, either in one of the fused cells or distributed between both. The cells may form protuberances by which conjugation takes place. Single spores give cultures which sporulate again (van der Walt and van der Klift 1972, Kurtzman and Kreger-van Rij 1976).

Ascospores were observed on V8 and YM agars after 1–2 weeks at or below 20°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	w/–
Galactose	s	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	w/–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	s
Soluble starch	w/–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	+/w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	s
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	10% NaCl/5% glucose	+
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Growth at 37°C	–

Co-Q: 9 (van der Walt and Johannsen 1980).
Mol% G+C: 39.8, CBS 6344 (BD: Price et al. 1978).

Origin of the strains studied: Digestive tract of the African honey bee (*Apis mellifera* var. *adansonii*), CBS 6344 = JCM 1707 = NRRL Y-7585; soil in South Africa, CBS 6694 = NRRL Y-7772 = JCM 6195; unknown, NRRL Y-7804 = JCM 1708.

Type strain: CBS 6344, isolated from an African bee.

26.8. *Debaryomyces nepalensis* S. Goto & Sugiyama (1968)

Anamorph: *Candida naganishii* Nakase & M. Suzuki
Synonyms:
Debaryomyces cavensis Giovannozzi (1941)
Debaryomyces japonicus Naganishi (1941a)
?*Saccharomyces disaccharomellis* Kawano, Kojima, Ohosawa & Morinaga (1976)
Candida naganishii Nakase & M. Suzuki (1985b)

Growth in YM broth: After 3 days at 25°C, the cells are spheroidal, sub-spheroidal, or short ovoidal, $(2–6)\times(2.5–6.5)$ µm, and single, in pairs, or in short chains. A sediment, a ring, and occasionally a thin, smooth or wrinkled pellicle or islets are formed.

Growth on YM agar: After one month at 17°C, the streak culture is grayish to yellowish, soft, shiny or dull, smooth or wrinkled.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudomycelium is absent or rudimentary.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. Spores are spheroidal and have a warty wall which was observed by Banno and Mikata (1985) using scanning electron microscopy. Usually, one spore is formed per ascus.

Ascospores were observed on YM agar after 1–2 weeks at or below 20°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	w/–
Sucrose	w/–	Trehalose	w/–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	v
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	w	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Growth at 37°C	–
Saccharate	–	Max. growth temp. (°C)	36–37
10% NaCl/5% glucose	+		

Co-Q: 9 (minor component 8) JCM 2095 and JCM 2164 (Nakase and Suzuki 1985a); CBS 5230 (Billon-Grand 1987).

Mol% G + C: 37.6–38.0, type strain and 3 additional strains (T_m: Nakase and Suzuki 1985a); 39.1 mol%, CBS 5921 (BD: Price et al. 1978).

Origin of the strains studied: Soil in Nepal, CBS 5921 = JCM 2095 = NRRL Y-7534; fermenting Maryland tobacco, CBS 1325 = JCM 2164, type strain of *D. cavensis;* sediment in spoiled sake, CBS 2334, type strain of *D. japonicus;* unknown, JCM 2209 = IFO 0085.

Type strain: CBS 5921.

Comments: *D. nepalensis* shows close physiological similarity to *D. hansenii,* but it is a genetically distinct species from *D. hansenii* (Price et al. 1978, Nakase and Suzuki 1985a). See Comments under *D. hansenii.*

26.9. *Debaryomyces occidentalis* (Klöcker) Kurtzman & Robnett (1991)

This species has two varieties:

Debaryomyces occidentalis (Klöcker) Kurtzman & Robnett var. *occidentalis* (1991)

Synonyms:
Schwanniomyces occidentalis Klöcker (1909a)
Schwanniomyces castellii Capriotti (1957)
Schwanniomyces alluvius Phaff, M.W. Miller & Cooke (1960)

Debaryomyces occidentalis var. *persoonii* (van der Walt) Kurtzman & Robnett (1991)

Synonyms:
Schwanniomyces persoonii van der Walt (1962)
Schwanniomyces ukrainicus Kvasnikov, Nagornaya & Shchelokova (1979a)
Schwanniomyces occidentalis Klöcker var. *persoonii* (van der Walt) Phaff & M.W. Miller (1984b)

Growth in YM broth: After 3 days at 25°C, the cells are ovoidal, globose, or sometimes egg-shaped, (3.5–8.0)×(4.5–9.0) µm, occasionally up to 10 µm long, and single, in pairs or in short chains or clusters. No pellicle is formed, but a sediment is present.

Growth on YM agar: After one month at 17°C, the streak culture is cream-colored, smooth, and semi-glossy; the texture is pasty to soft. The cross section is broadly convex and the border entire.

Slide culture on corn meal agar: Pseudomycelium is absent aerobically and anaerobically.

Formation of ascospores: Cells are haploid. Before sporulation the nucleus divides, which is followed by fusion. The diploid nucleus ordinarily undergoes reduction division in a special open bud-like structure, the meiosis bud, and this is followed by the formation of one or, more rarely, two ascospores in the parent cell. Alternatively, the nucleus in the parent cell may divide, after which one of the daughter nuclei moves into a small bud which is temporarily separated from the parent cell by a septum. The septum then lyses, the two nuclei fuse, and meiosis follows. Rarely, diploidization is accomplished by the fusion of two individual cells. The spores are globose, have a warty surface, an equatorial ledge and a pronounced lipid globule. They somewhat resemble the appearance of a walnut. There is usually one spore per ascus, occasionally two (Fig. 52).

Sporulation is best after approximately 5–7 days on yeast autolyzate agar with 2% glucose. Ascospores are also observed on YM agar after 1–2 weeks at 25°C.

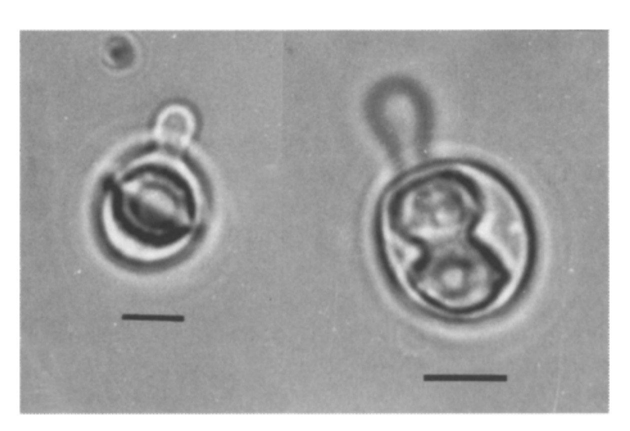


Fig. 52. *D. occidentalis* var. *occidentalis,* CBS 4516. Asci with one or two ascospores, after one week on yeast autolyzate agar with 2% glucose at 25°C. Bars = 4 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	w/–
Sucrose	+	Trehalose	–
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	s
Melibiose	v	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	s
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	v	Citrate	s
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	v
L-Rhamnose	–	Nitrate	–
D-Glucosamine	ws	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	–
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Growth at 37°C	v
7.5% NaCl/5% glucose	+		

Co-Q: 9, CBS 819 (Yamada et al. 1977).

Mol% G+C: 35.2, CBS 4516, type strain of *Schwanniomyces alluvius;* 35.2, CBS 2863, type strain of *Schwanniomyces castellii;* 35.3, CBS 1153, Carlsberg strain of *Schwanniomyces occidentalis* (BD: Price et al. 1978); 35.4, variety *persoonii* (BD: Price et al. 1978).

Supplementary description of *D. occidentalis* var. *persoonii*: The variety *persoonii* does not assimilate cellobiose, galactose or D-xylose and these differences allow it to be separated from the variety *occidentalis* using standard growth tests. In addition, the variety *persoonii* does not grow on salicin, or on 7.5% NaCl/5% glucose. Morphologically the two varieties are similar, as are their life cycles.

Origin of the strains belonging to the variety *occidentalis*: Soil samples in Ohio, Michigan, Spain, Island of St. Thomas (10).

Type strain: CBS 819 (ATCC 2322).

Origin of the strains belonging to the variety *persoonii*: Soil samples in the Transvaal, South Africa (2), Russia (1).

Type strain: CBS 2169 (NRRL Y-7400).

Comments: Kurtzman and Robnett (1991) proposed the transfer of *Schwanniomyces occidentalis* to the genus *Debaryomyces* on the basis of sequence similarity in partial ribosomal RNA sequences between *S. occidentalis* and several *Debaryomyces* spp. They determined the extent of divergence in three regions from 18S and 25S subunit ribosomal RNAs, comprising a total of 900 nucleotides.

D. occidentalis can be distinguished from other ascomycetous yeast species by the unique shape of the ascospores. Kurtzman et al. (1972) have illustrated the spore topography by scanning electron microscopy. Ferreira and Phaff (1959) described the life cycle of *Schwanniomyces* based on nuclear staining of cells in the process of meiosis. They presented evidence that, following karyogamy, meiosis took place in an open bud-like structure, the meiosis bud, and that the haploid nuclei moved back into the parent cell, where one, or more rarely two, developed into ascospores. Kreger-van Rij (1977a) restudied the life cycle and concluded that conjugation took place between a small bud cell and the parent cell by lysis of the connecting septum and that karyogamy and meiosis probably occur in the parent cell. This view was shared by Forrest et al. (1987). It is also possible that both types of life cycles mentioned above occur in this yeast. Kreger-van Rij (1977a) also determined, by electron microscopy of ultrathin sections, details of spore wall development and internal structure.

Price et al. (1978) carried out DNA/DNA reassociation experiments among the various strains of *Schwanniomyces.* Polynucleotide sequence relationships between reference DNA from *S. occidentalis* and DNA from *S. castellii* and *S. alluvius* were at the 97% level or greater and the last two species were therefore placed into synonymy with *S. occidentalis.* The type strain of *S. persoonii* showed 83% and a second strain 80% sequence complementarity with the DNA from *S. occidentalis.* This lower DNA relatedness coupled with several differences in carbon compound assimilation induced these authors to retain *S. persoonii* as a variety of *S. occidentalis.*

Yamada et al. (1991c) reported partial sequences of 18S and 26S ribosomal RNAs of strains of *S. occidentalis* var. *occidentalis* and *S. occidentalis* var. *persoonii.* They suggested that the genus *Schwanniomyces* might be retained as its own genus separate from the genus *Debaryomyces* based on the two base differences observed in 168 bases of 18S RNA between the type species of the genera. However, since the two base differences are considered to be too small for generic separation, we adopted the proposal by Kurtzman and Robnett (1991).

26.10. *Debaryomyces polymorphus* (Klöcker) Price & Phaff (1979)

Synonyms:

Pichia polymorpha Klöcker (1912a)
Saccharomyces polymorphus (Klöcker) Novák & Zsolt (1961)
Torulaspora polymorpha (Klöcker) van der Walt & E. Johannsen (1975a)
Debaryozyma polymorpha (Klöcker) van der Walt & E. Johannsen (1978)
Debaryomyces cantarellii Capriotti (1961a)
Pichia cantarellii (Capriotti) Campbell (1973)
Debaryomyces phaffii Capriotti (1961b)
Torulaspora phaffii (Capriotti) van der Walt & E. Johannsen (1975a)

Growth in YM broth: After 3 days at 25°C, the

cells are ovoidal, long-ovoidal and cylindroidal, (1.5–7.5)×(3.5–13.5) µm and longer; they are single or in pairs. A sediment and a thin pellicle are formed.

Growth on YM agar: After one month at 17°C, the streak culture is cream-colored to yellowish, soft, flat, smooth or partly wrinkled, dull to shiny.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed. Tree-like chains of cells may be present.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. Conjugation between two cells of nearly equal size may also occur. The spores are spheroidal with a warty wall. The warts are not always distinct with the light microscope. With scanning electron microscopy they are clearly observed (Kurtzman and Smiley 1974, Kurtzman et al. 1975). Asci may form up to two spores, but there is generally one spore per ascus.

Ascospores were observed on V8, Gorodkowa and corn meal agars after 1–2 weeks at or below 20°C.

Fermentation:

Glucose	s	Lactose	–
Galactose	w/–	Raffinose	ws
Sucrose	s	Trehalose	–
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	v
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	+
5-Keto-D-gluconate	v	Starch formation	–
Saccharate	–	Growth at 37°C	v

Co-Q: 9 (minor components 7, 8 and 10) (Yamada et al. 1973a, 1976b, Billon-Grand 1987).

Mol% G+C: 35.7–35.9, 5 strains (BD: Price et al. 1978).

Origin of the strains studied: Soil in Denmark, CBS 186 = JCM 3647, CBS 1359; soil in the Netherlands (1); soil in Finland, CBS 4346, type strain of *Debaryomyces phaffii;* CBS 4349, type strain of *Debaryomyces cantarellii;* CBS 4350; toadstool (1); anthill in Russia (2).

Type strain: CBS 186, from Klöcker's collection.

Comments: Forrest et al. (1987) studied the nuclear behavior in growing, dividing, and ascospore-forming cells of a strain of *D. polymorphus* by light microscopy of fixed Giemsa stained preparations. They concluded that meiosis took place in the parent cell and not in the bud, and that the term "meiosis bud" therefore did not apply to this yeast.

26.11. *Debaryomyces pseudopolymorphus* (C. Ramírez & Boidin) Price & Phaff (1979)

Synonyms:

Pichia pseudopolymorpha C. Ramírez & Boidin (1953a)
Saccharomyces pseudopolymorphus (C. Ramírez & Boidin) Novák & Zsolt (1961)
Torulaspora pseudopolymorpha (C. Ramírez & Boidin) van der Walt & E. Johannsen (1975a)
Debaryozyma pseudopolymorpha (C. Ramírez & Boidin) van der Walt & E. Johannsen (1978)

Growth in YM broth: After 3 days at 25°C, the cells are long-ovoidal to cylindroidal, (3–6.5)×(4.5–16) µm, and single or in pairs. A sediment and a dull creeping pellicle are formed.

Growth on YM agar: After one month at 17°C, the streak culture is white or cream-colored to yellowish, soft, dull and wrinkled. The edge is fringed with pseudomycelium.

Dalmau plate culture on corn meal agar: A primitive pseudomycelium is formed that consists of tree-like chains of elongated cells.

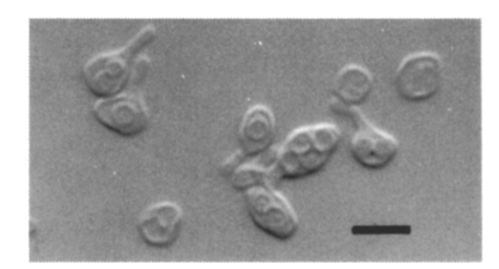


Fig. 53. *D. pseudopolymorphus*, CBS 2008. Asci with ascospores, after one month on V8 agar at 20°C. Bar = 5 µm.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. The spores are spheroidal. They have a warty wall which is distinct in the scanning electron microscope (Kurtzman and Smiley 1974), but not always visible under the light microscope. One to four spores are formed per ascus (Fig. 53). According to Kreger-van Rij (1984d), one to two spores form on Gorodkowa agar and two to four on acetate agar, but we observed one to four on Gorodkowa agar.

Ascospores were observed on acetate, Gorodkowa and V8 agars after 1–2 weeks at or below 20°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	ws	Raffinose	–
Sucrose	s	Trehalose	–
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	w/–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	+
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Growth at 37°C	–

Co-Q: 9 (minor components 7, 8 and 10) (Yamada et al. 1973a, Billon-Grand 1987).

Mol% G+C: 35.7, 2 strains (BD: Price et al. 1978).

Origin of the strains studied: Tanning liquor, CBS 2008, CBS 2009 = JCM 6184.

Type strain: CBS 2009.

26.12. *Debaryomyces robertsiae* (van der Walt) Kurtzman & Robnett (1994b)

Synonyms:

Pichia robertsiae (as *P. robertsii*) van der Walt (1959a)
Wingea robertsii (van der Walt) van der Walt (1967) nom. inval.
Wingea robertsii (van der Walt) van der Walt (1971)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal (2.1–7.0)×(2.6–7.0) µm, and occur singly, in pairs or occasionally in small clusters. Growth is butyrous and light tannish-white in color.

Growth on the surface of assimilation media: Dry climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, only budding yeast cells are detected under the coverglass. Aerobic growth is white, butyrous, and with a smooth semi-glistening surface having a depressed center. Colony margins are smooth to finely scalloped. A faint acidic odor is present.

Formation of ascospores: Asci are occasionally unconjugated, but most show conjugation between independent cells or between a cell and its bud. Some asci form protuberances which may serve the function of bud conjugants. Asci are persistent and contain one to four ascospores; the ascospores are smooth, lenticular and show a light brown coloration (Figs. 54, 55). Heavily sporulating cultures become dark red in color. Single-spore isolates obtained by micromanipulation of random spores freed enzymatically from asci of NRRL Y-7680 (CBS 5637) gave only sporogenous colonies suggesting that *D. robertsiae* is homothallic (Kurtzman, unpublished data).

Ascospores were observed on YM agar after 1–2 weeks at 25°C. Sporulation is often sparse but some single-spore isolates sporulate heavily as do occasional colony sectors.

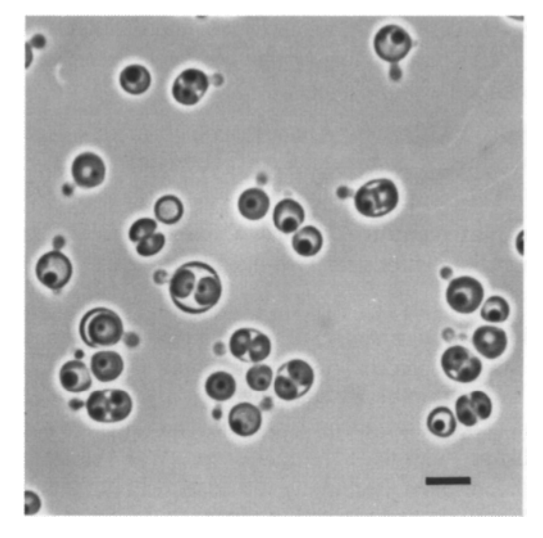


Fig. 54. *D. robertsiae,* CBS 5637. Asci with 1–2 ascospores, after 1 week on YM agar at 25°C. Bar = 5 µm.

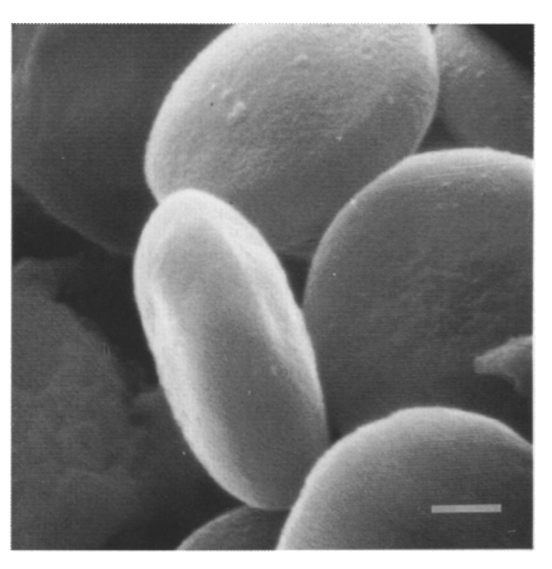


Fig. 55. *D. robertsiae,* CBS 5637. Scanning electron micrograph of enzymatically freed ascospores. Bar = 0.5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w/–
Sucrose	+	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	+/w
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	+/s

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 2934 (Yamada et al. 1973a).
Mol% G + C: 42.7, CBS 2934 (BD: Price et al. 1978).

Origin of the strains studied: NRRL Y-6670 (CBS 2934), from a larval feed of the South African carpenter bee *(Xylocopa caffra)*, Transvaal; NRRL Y–7680 (CBS 5637), from pollen carried by a South African carpenter bee, Transvaal.

Type strain: CBS 2934 (NRRL Y-6670), from van der Walt, South Africa.

Comments: *D. robertsiae* is unique among yeasts because of the lenticular shape of its ascospores, otherwise, it is phenotypically similar to other members of the genus *Debaryomyces*. Kurtzman and Robnett (1994b) showed from analysis of partial 18S and 26S rRNA sequences that *D. robertsiae* is a member of the genus *Debaryomyces*.

26.13. *Debaryomyces udenii* van der Walt, M.Th. Smith & Y. Yamada (1989b)

Growth in YM broth: After 3 days at 25°C, the cells are spheroidal to short-ovoidal (2.0–5.6) × (2.5–5.6) μm, and single, in pairs, in short chains or in small clusters. A sediment and a thin pellicle are formed.

Growth on YM agar: After one month at 17°C, the streak culture is cream-colored, soft, smooth and dull.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. Asci also arise from the conjugation of independent cells. Asci slowly lyse, liberating the ascospores which tend to agglutinate. The ascospores are globose to ellipsoidal, and appear glabrous by light microscopy. By TEM, the surface appears to be colliculate to pusticulate (van der Walt et al. 1989b). One

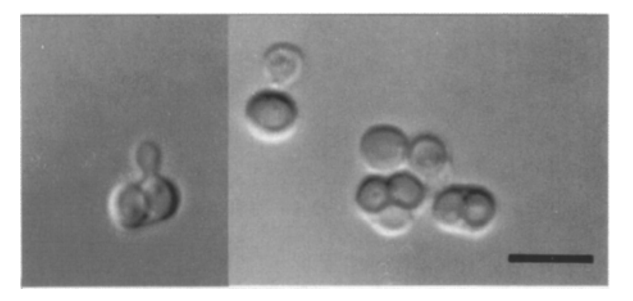


Fig. 56. *D. udenii*, CBS 7056. Lysing asci with ascospores, after two weeks on YM agar at 25°C. Bar = 5 μm.

to four spores per ascus are formed (Fig. 56). Actively sporulating cultures on YM agar are pale brown.

Ascospores were observed on YM agar after 1–2 weeks at 25°C.

Fermentation:

Glucose	ws	Lactose	–
Galactose	–	Raffinose	–
Sucrose	w/–	Trehalose	w/–
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	ws	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	s	Citrate	s
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	s
L-Rhamnose	ws	Nitrate	–
D-Glucosamine	w/–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	+
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Growth at 37°C	–

Co-Q: 9, CBS 7056 (van der Walt et al. 1989b).
Mol% G + C: 35.8, CBS 7056 (T_m: van der Walt et al. 1989b).

Origin of the strains studied: Soil samples collected in the Pinery Provincial Park, Ontario, Canada (CBS 7056 and CBS 7057).

Type strain: CBS 7056.

Comments: The results from the present study are in agreement with the original description, except for the positive assimilation of soluble starch. *D. udenii* forms colliculate ascospores which can be liberated from the ascus. By comparison of 18S and 26S ribosomal RNA partial sequence analyses, Yamada et al. (1991a) showed that *D. udenii* is closely related to other *Debaryomyces* species. *D. udenii* physiologically resembles *D. castellii*,

D. pseudopolymorphus, and *D. yamadae,* but it is distinguished from these three species by its lytic asci and by differences in the assimilation of lactose, melibiose and erythritol.

26.14. *Debaryomyces vanrijiae* (van der Walt & Tscheuschner) Abadie, Pignal & J.L. Jacob (1963)

This species has two varieties:

Debaryomyces vanrijiae (van der Walt & Tscheuschner) Abadie, Pignal & J.L. Jacob var. *vanrijiae* (1984)

Synonyms:

Pichia vanrijiae (as *P. vanriji*) van der Walt & Tscheuschner (1956a)
Azymomyces vanriji (van der Walt & Tscheuschner) Novák & Zsolt (1961)
Torulaspora vanriji (van der Walt & Tscheuschner) van der Walt & E. Johannsen (1975a)
Debaryozyma vanriji (van der Walt & Tscheuschner) van der Walt & E. Johannsen (1978)
Debaryomyces konokotinae Kudryavtsev (1960)
Debaryomyces formicarius Golubev & Bab'eva (1972)
Torulaspora formicaria (Golubev & Bab'eva) van der Walt & E. Johannsen (1975a)

Debaryomyces vanrijiae var. *yarrowii* (Santa María & García Aser) Kreger-van Rij (1984d)

Synonyms:

Debaryomyces yarrowii Santa María & García Aser (1971)
Torulaspora yarrowii (Santa María & García Aser) van der Walt & E. Johannsen (1975a)

Growth in YM broth: After 3 days at 25°C, the cells are spheroidal to short-ovoidal (3–8.5)×(3–20) μm, and single or in groups. A sediment and a white, wrinkled, creeping pellicle are formed.

Growth on YM agar: After one month at 17°C, the streak culture is yellow to yellowish-brown, dull or semi-glistening, and smooth with a sinuous margin.

Dalmau plate culture on corn meal agar: A primitive pseudomycelium is formed that consists of short elongated cells.

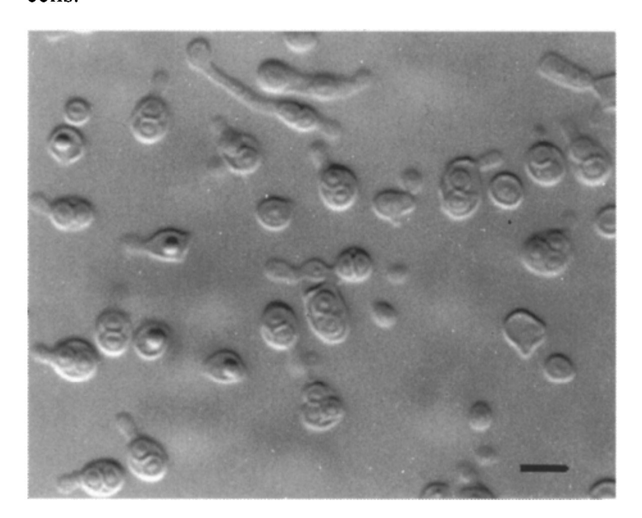


Fig. 57. *D. vanrijiae* var. *vanrijiae,* CBS 3024. Asci with ascospores, after one month on V8 agar at 20°C. Bar = 5 μm.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. The spores are spheroidal, and one to four are formed per ascus (Fig. 57). They have a warty wall which is not always visible under the light microscope, but is clearly observed by scanning electron microscopy (Kurtzman et al. 1975) and in ultrathin sections (Santa María and García Aser 1971).

Ascospores were observed on V8, acetate, YM and malt agars after 1–2 weeks at or below 20°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	w/–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	v	D-Gluconate	+
Soluble starch	+	DL-Lactate	w/–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	+
L-Rhamnose	v	Nitrate	–
D-Glucosamine	v	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	+
5-Keto-D-gluconate	v	Starch formation	–
Saccharate	–	Growth at 37°C	+

Co-Q: 9 (minor component 8) (Yamada et al. 1976b, Billon-Grand 1987).

Mol% G + C: 33.2–33.3, 4 strains (Price et al. 1978); 31.7, CBS 3024 (T_m: Suzuki and Nakase, unpublished data).

Supplementary description of *D. vanrijiae* var. *yarrowii*: By the addition of one strain assigned to the variety *yarrowii,* the description given by Kreger-van Rij (1984d) is changed as follows:

Assimilation of carbon compound:

Trehalose	w/–	Salicin	v
Melibiose	–	Citric acid	v
Soluble starch	v	Growth at 37°C	–
Erythritol	v		

Mol% G + C: 33.0, CBS 6246 (BD: Price et al. 1978); 32.1, CBS 6246; 30.6, CBS 6755 (T_m: Suzuki and Nakase, unpublished data).

Origin of the strains belonging to the variety *vanrijiae*: Soil in South Africa, CBS 3024; exudate of slippery-elm tree, CBS 5458; anthill, CBS 6454, type strain of *Debaryomyces formicarius,* CBS 6455; unknown, CBS 5709, CBS 6756.

Type strain: CBS 3024, isolated by van der Walt from soil in South Africa.

Origin of the strains belonging to the variety *yarrowii*: Excrements of insects on *Paulownia imperialis* in Madrid, CBS 6246; unknown, CBS 6755.

Type strain: CBS 6246.

Comments: Price et al. (1978) found the type strains of *D. yarrowii* and *D. vanrijiae* to show 68.4% DNA base sequence complementarity. They maintained them as separate species, whereas Kreger-van Rij (1984d) reclassified *D. yarrowii* as a variety of *D. vanrijiae*, based on the view by Kurtzman et al. (1980b) that this percentage relatedness may indicate that the two strains belong to the same species. In this study, our DNA–DNA hybridization by membrane filter method showed 74% DNA relatedness between the type strains of *D. yarrowii* and *D. vanrijiae*. A single strain, CBS 6755, which has intermediate physiological properties between the two species, showed 87% DNA relatedness to the type strain *D. yarrowii* and 78% DNA relatedness to the type strain of *D. vanrijiae* (Suzuki and Nakase, unpublished data). Based on the view of Kurtzman (1987b) that DNA relatedness of 65–70% or greater suggests strains to be conspecific in most comparisons, we accepted Kreger-van Rij's classification. Also, we assigned strain CBS 6755 to the variety *yarrowii*, and hence the physiological differences between the two varieties were reduced. The variety *yarrowii* may still be distinguished from the variety *vanrijiae* by its inability to assimilate melibiose and lack of growth at 37°C.

26.15. *Debaryomyces yamadae* (van der Walt & E. Johannsen) van der Walt, M.Th. Smith & Y. Yamada (1989b)

Synonyms:

Debaryozyma yamadae van der Walt & E. Johannsen (1980)

Debaryomyces yamadae (van der Walt & E. Johannsen) J.A. Barnett, Payne & Yarrow (1990)

Growth in YM broth: After 3 days at 25°C, the cells are spheroidal, ovoidal or ellipsoidal (1.5–5.0)×(2.0–6.0) μm; they may be single, in pairs, short chains or in small clusters. A sediment and a slight ring are formed.

Growth on YM agar: After one month at 17°C, the streak culture is cream-colored or brownish and shiny. The margin is entire to undulating.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed.

Formation of ascospores: Conjugation between a cell and its bud precedes ascus formation. The spores are spheroidal and warty with one to four spores per ascus. The wartiness is not always visible under the light microscope, but is detectable in a ultrathin section (van der Walt and Johannsen 1980).

Ascospores were observed on YM agar and malt extract agar after 1–2 weeks at or below 20°C.

Fermentation:

Glucose	s	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	s	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	w
Soluble starch	w/–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	v
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Growth at 37°C	–

Co-Q: 9 (van der Walt and Johannsen 1980).

Mol% G+C: 34.5, CBS 7035; 35.7, CBS 7036 (T_m: Suzuki and Nakase, unpublished data).

Origin of the strains studied: Soil, CBS 7035; arboricolous lichen, CBS 7036.

Type strain: CBS 7035.

Comments: Results from the present study are in agreement with the original description of this species, which was described as *Debaryozyma yamadae* by van der Walt and Johannsen. As a result of the acceptance of *Debaryomyces* Lodder & Kreger-van Rij nom. cons. during the International Botanical Congress (1987), the genus *Debaryozyma* van der Walt & Johannsen became a synonym of the genus *Debaryomyces*. For this reason, *Debaryomyces yamadae* is the valid name of this species. *Debaryomyces yamadae* (van der Walt & E. Johannsen) van der Walt, M.Th. Smith & Y. Yamada (1989b) has priority over *Debaryomyces yamadae* (van der Walt & E. Johannsen) J.A. Barnett, Payne & Yarrow (1990).

Comments on the genus

Lodder and Kreger-van Rij (1978) made an official proposal for conservation of the name *Debaryomyces* Lodder & Kreger-van Rij against *Debaryomyces* Klöcker. The type species of the latter, *Debaryomyces globosus* Klöcker, had been classified in the genus *Saccharomyces* (van der Walt 1970d), and it is now classified in the genus *Torulaspora*. After publication of this proposal, van der Walt and Johannsen (1978) proposed a new

name, *Debaryozyma*, for *Debaryomyces* species. However, in accordance with Recommendation 15A of the International Code of Botanical Nomenclature, the name *Debaryomyces* was applicable pending a decision on the proposal by the General Committee (Lodder and Kreger-van Rij 1979). Finally, in the 1988 International Code of Botanical Nomenclature (Greuter et al. 1988), the proposal by Lodder and Kreger-van Rij was accepted. Because *Schwanniomyces occidentalis* has nomenclatural priority over extant species of *Debaryomyces*, Kurtzman is submitting a proposal to conserve *Debaryomyces* against *Schwanniomyces*.

The genus *Debaryomyces* Lodder & Kreger-van Rij (1952) (Kreger-van Rij 1970a, 1984d) is distinguished from other ascomycetous yeast genera by the special structure of the spore wall (wartiness), multilateral budding, inability to assimilate nitrate, coenzyme Q-9, and fermentation that is absent, weak or vigorous. All *Debaryomyces* species are haploid and conjugation between a cell and its bud generally precedes ascus formation. Accompanied with the transfer of *Schwanniomyces occidentalis* to *Debaryomyces*, Kurtzman and Robnett (1991) emended the diagnosis of the genus *Debaryomyces* as follows: Ascospores have walls with warts that may also include the presence of ridges or an equatorial ring. Fermentation is absent, weak, or occasionally vigorous. In this edition, the generic description is emended further by the inclusion of *Debaryomyces udenii* which has lysing asci.

Kreger-van Rij and Veenhuis (1975a) described the internal ultrastructure of the spore wall of *D. hansenii* which is, as far as is known, also typical of the other *Debaryomyces* species. Spheroidal warty spores in other nitrate-negative genera, such as *Torulaspora*, *Saccharomyces* and *Zygosaccharomyces*, have, with the exception of *Zygosaccharomyces rouxii* (Kreger-van Rij 1979), a different wall structure. *Issatchenkia* species also have warty spores, but the asci are generally unconjugated. *Saccharomyces*, *Zygosaccharomyces*, *Torulaspora* and *Issatchenkia* differ from *Debaryomyces* in the number of isoprene units of the major coenzyme Q: *Saccharomyces*, *Zygosaccharomyces*, and *Torulaspora*, 6; *Issatchenkia*, 7; and *Debaryomyces*, 9 (Yamada et al. 1973a, 1976b, Nakase and Suzuki 1985a, Billon-Grand 1987).

A proposal by van der Walt and Johannsen (1975a) to combine *Debaryomyces* and *Torulaspora* species in the genus *Torulaspora* was rejected by Kreger-van Rij and Veenhuis (1976b). A distinct difference in the structure of the spore wall between *Debaryomyces* and *Torulaspora* species correlates with other differences between them. Price et al. (1978) gave additional arguments for keeping *Debaryomyces* separate from the *Torulaspora* species. Kocková-Kratochvílová et al. (1978) applied numerical taxonomy to *Debaryomyces* species and species of the *Torulaspora* group. The latter were clearly distinguishable from the *Debaryomyces* species.

Price et al. (1978) made an important study of DNA sequence relatedness among *Debaryomyces* and several similar *Pichia* species which clarified taxonomic relations and resulted in merging of some species, e.g., *D. cantarellii* and *D. phaffii* with *Pichia polymorpha* to become *D. polymorphus*.

It is noted that, with respect to its ascosporal surface structure, *D. udenii* is intermediate between smooth round spore-forming *Pichia* species possessing ubiquinone Q-9 (*P. tannicola*, *P. carsonii* and *P. etchellsii*) and other *Debaryomyces* species which form warty ascospores. Billon-Grand (1988, 1989 and personal communication) suggested the transfer of *P. abadieae*, *P. carsonii*, *P. etchellsii* and *P. humboldtii* to the genus *Debaryomyces* because they show the same major ubiquinone Q-9, similar intracellular oxidases and vitamin requirements, and produce round ascospores. Recently, Yamada et al. (1992b) reported the partial sequences of 18S and 26S ribosomal RNAs of the above four *Pichia* species, and suggested that *P. carsonii* and *P. etchellsii* have phylogenetically close relationships to *Debaryomyces* species and that *P. abadieae* (= *P. tannicola*) and *P. humboldtii* are phylogenetically distant from each other and from *P. carsonii*, *P. etchellsii* and *Debaryomyces* species. De Hoog et al. (1986) regarded *P. humboldtii* as a synonym of *Dipodascus ingens*. Consequently, it seems reasonable to transfer *P. carsonii* and *P. etchellsii* to the genus *Debaryomyces* along with emendation of the generic description to permit inclusion of species forming smooth spheroidal ascospores. By this emendation, however, wartiness of ascospores becomes less significant as a criterion for generic delimitation of the genus *Debaryomyces*.

Species not accepted in the genus

***Debaryomyces tamarii* Ohara & Nonomura (1954d):** This species no longer forms ascospores and has several exceptional features among members of the genus *Debaryomyces*. Ribosomal RNA sequence comparisons by Kurtzman and Robnett (1991) showed *D. tamarii* to be well separated from other species of *Debaryomyces*. Its G + C content of nuclear DNA is much higher than that of the other *Debaryomyces* species (Nakase and Komagata 1971a, Price et al. 1978). We also found in this study that the assimilation of nitrate of the type strain was positive within one week after inoculation, and that its whole cell hydrolyzate contained glucose, mannose and galactose whereas those of other *Debaryomyces* species contained glucose and mannose (Suzuki and Nakase, unpublished data). For these reasons, this species is no longer maintained in the genus *Debaryomyces*. F.-L. Lee et al. (1992) and Suzuki et al. (1992) independently demonstrated that this species was conspecific with *Candida versatilis* based on DNA–DNA hybridization.

***Debaryomyces artagaveytiae* Batista, Silveira & Coelho (1960):** Ribosomal RNA sequence comparisons by Kurtzman and Robnett (1991) showed that strain CBS 5285 (=NRRL YB-4906) represented a species so

divergent that its assignment to *Debaryomyces* could no longer be maintained. In this study, we found strain CBS 5285 to be urease positive and DBB positive, and that it contained glucose, mannose, galactose and fucose in acid hydrolyzates. D. Yarrow (personal communication) also found CBS 5285 to hydrolyze urea and to be DBB positive, and suggested a relationship with *Sterigmatomyces*. Furthermore, strain CBS 5285 is not the type; the type strain of this species (IMUR 2201) is not available at present.

27. *Dekkera* van der Walt

M.Th. Smith

Diagnosis of the genus

Budding cells are spheroidal, subglobose to ellipsoidal, frequently ogival, or cylindroidal to elongate. Pseudomycelium and branched, one-celled, non-septate mycelium are sometimes formed.

Asci arise without conjugation, are evanescent, and form 1–4 ascospores. Ascospores are hat-shaped or somewhat spheroidal with tangential brims, and tend to agglutinate when released.

Cultures are slow growing and generally short-lived. Acetic acid is produced aerobically from glucose. Fermentation is usually stimulated by molecular oxygen. An extraneous vitamin source is required. Diazonium blue B reaction is negative.

Type species

Dekkera bruxellensis van der Walt

Species accepted

1. *Dekkera anomala* M.Th. Smith & van Grinsven (1984)
2. *Dekkera bruxellensis* van der Walt (1964)

Key to species

See Table 21.

1. a D-Glucitol assimilated → 2
   b D-Glucitol not assimilated → 3

2(1). a Succinate assimilated .... *Brettanomyces naardenensis*: p. 451
   b Succinate not assimilated .... *Brettanomyces nanus*: p. 452

3(1). Species with the following combinations of characters:
   a Slender, often branched, non-septate filaments are produced on Dalmau plates; lactose fermented by most strains; succinate latently assimilated by most strains; galactose assimilated .... *Dekkera anomala*: p. 174
   b Non-septate filaments are not produced; lactose not fermented; succinate not assimilated; galactose assimilation variable .... *Dekkera bruxellensis*: p. 175
   c Non-septate filaments are not produced; lactose not fermented; succinate assimilated; galactose not assimilated .... *Brettanomyces custersianus*: p. 451

Table 21
Key characters of species in the genus *Dekkera* and its anamorph *Brettanomyces*

Species	Lactose fermentation	Assimilation			Non-septate filaments
		Galactose	Glucitol	Succinate	
Dekkera anomala	v	+	–	v	+
D. bruxellensis	–	v	–	–	–
Brettanomyces custersianus	–	–	–	+	–
B. naardenensis	–	+	+	+	–
B. nanus	–	+	+	–	–

Systematic discussion of the species

27.1. *Dekkera anomala* M.Th. Smith & van Grinsven (1984)

Anamorph: *Brettanomyces anomalus* Custers

Synonyms:

Monilia vini Osterwalder (1912)
Oospora vini (Osterwalder) Janke (1924)
Brettanomyces anomalus Custers (1940)
Brettanomyces claussenii Custers (1940)
Dekkera claussenii F.-L. Lee & S.-C. Jong (1985)
Torulopsis cylindrica Walters (1943)
Brettanomyces claussenii Custers var. *sablieri* Legakis (1961) nom. inval.
Brettanomyces cidri Legakis (1961)
Brettanomyces dublinensis Gilliland (1962)
Candida beijingensis Yue & Pna (1984)

Growth in malt extract: After 5 days at 25°C, the cells are spheroidal, ellipsoidal, ogival or cylindroidal to elongate (2.0–5.5)×(3.0–22.0) µm. Cells occur singly, in pairs, short chains or clusters. Pseudomycelium is usually formed; it is filamentous, and frequently branching, but non-septate cells are also produced. A floccose to mucoid sediment is formed. Pellicle formation is absent.

Growth on malt agar + 2% calcium carbonate: After 6 weeks at room temperature, the streak culture is

restricted, slightly raised in the center, white to cream-colored or creamish-brown, somewhat shiny to dull, and smooth or rugose. Margins are entire, lobate or fringed with mycelium. Acetic acid is produced.

Dalmau plate cultures on potato-, rice- and morphology agars: After 5 days at 25°C, pseudomycelium is produced, and consists of filamentous cells that form ellipsoidal to cylindroidal blastoconidia that occur singly or in short chains. Slender, non-septate filaments are present and they are often branched (Fig. 58).

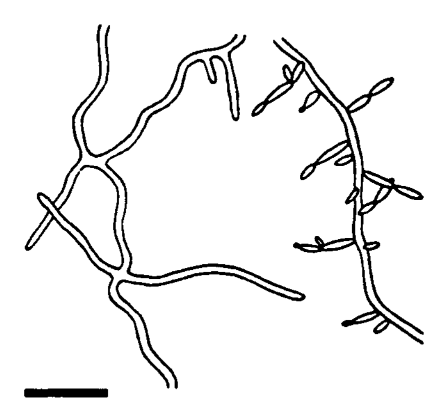


Fig. 58. *D. anomala.* Filaments on corn meal agar after 10 days at 25°C. Bar = 25 μm (from van der Walt 1970e).

Formation of ascospores: Vegetative cells are directly transformed into evanescent asci without preceding conjugation. One to four galeate ascospores are formed per ascus and agglutinate when liberated.

Ascospore formation, which may be sparse, is observed on 3% Difco malt agar after 3 weeks at 25°C.

Fermentation:

Glucose	+	Lactose	v
Galactose	+	Raffinose	v
Sucrose	+	Trehalose	v
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	v
D-Glucosamine	+/w	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	–	0.1% Cycloheximide	v
0.01% Cycloheximide	+	Growth at 37°C	+

Co-Q: 9 (Yamada et al. 1980, Billon-Grand 1987).

Mol% G+C: 38.1–41.7, 10 strains, including CBS 76, CBS 77, CBS 1947, CBS 4460, CBS 5111, CBS 7250, CBS 8138, CBS 8139 (T_m: Smith et al. 1990b).

Origin of the strains studied: CBS 8139, spoiled soft drink, van Grinsven; CBS 76, beer, Kufferath, type strain of *B. claussenii*; CBS 77, stout, Custers, type strain of *B. anomalus*; CBS 1947, beer, Walters, authentic strain of *T. cylindrica*; CBS 4210, cider, Bidan, type strain of *B. cidri*; CBS 4460, cider, Bidan, type strain of *B. claussenii* var. *sablieri*; CBS 4608, sherry vat, van der Walt; CBS 5111, beer, Gilliland, authentic strain of *B. dublinensis*; CBS 7250, apple, Yue & Pna, type strain of *C. beijingensis*; 4 additional strains isolated from cider (3) and spoiled soft drink (1).

Type strain: CBS 8139, isolated by van Grinsven.

Comments: *Dekkera anomala* is considered the teleomorph of *Brettanomyces anomalus* on the basis of its morphological and physiological resemblance (Smith and van Grinsven 1984). Smith et al. (1990b) confirmed the proposed teleomorph–anamorph relationship from enzyme comparisons and DNA reassociations, and also demonstrated the synonymy of *B. cidri, B. claussenii, B. claussenii* var. *sablieri, B. dublinensis, Candida beijingensis* and *Torulopsis cylindrica* with *Dekkera anomala.*

27.2. *Dekkera bruxellensis* van der Walt (1964)

Anamorph: *Brettanomyces bruxellensis* Kufferath & van Laer

Synonyms:

Brettanomyces bruxellensis Kufferath & van Laer (1921)
Brettanomyces lambicus Kufferath & van Laer (1921)
Mycotorula intermedia Krumbholz & Tauschanoff (1933)
Brettanomyces intermedia (Krumbholz & Tauschanoff) van der Walt & van Kerken (1959) nom. inval.
Brettanomyces intermedius (Krumbholz & Tauschanoff) van der Walt & van Kerken (van der Walt 1971)
?*Brettanomyces bruxellensis* Kufferath & van Laer var. *lentus* Custers (1940)
Brettanomyces bruxellensis Kufferath & van Laer var. *nonmembranaefaciens* Custers (1940)
Brettanomyces custersii Florenzano (1950)
Brettanomyces patavinus Florenzano (1951) nom. inval.
Brettanomyces bruxellensis Kufferath & van Laer var. *vini* Barret, Bidan & André (1955) nom. inval.
Brettanomyces vini (Barret, Bidan & André) Peynaud & Domercq (1956) nom. inval.
Brettanomyces schanderlii Peynaud & Domercq (1956) nom. inval.
Dekkera intermedia van der Walt (1964)
Brettanomyces abstinens Yarrow & Ahearn (1971)
Dekkera abstinens F.-L. Lee & S.-C. Jong (1986b)
Dekkera lambica F.-L. Lee & S.-C. Jong (1986a)

Growth in malt extract: After 5 days at 25°C, the cells are ovoidal, ellipsoidal, frequently ogival or cylindroidal to elongate (2.0–7.0)×(3.5–28.0) μm. Cells occur singly, in pairs, short chains or small clusters (Fig. 59). Pseudomycelium is usually abundantly produced. A coherent

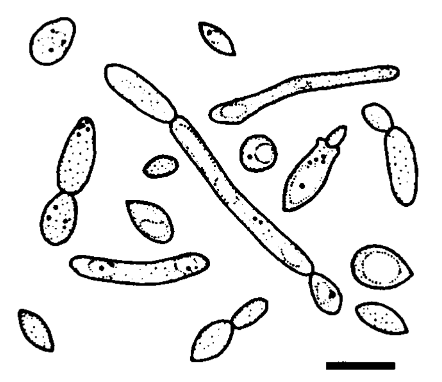


Fig. 59. *D. bruxellensis.* Budding cells in malt extract after 5 days at 25°C. Bar = 10 µm (from van der Walt 1970b).

floccose to somewhat mucoid sediment is formed; a slight ring is usually present and sometimes a creeping pellicle.

Growth on malt agar + 2% calcium carbonate: After 6 weeks at room temperature, the streak culture is usually restricted, raised, cream-colored to brownish-cream, shiny, smooth, crispulate or rugose. The margin is entire, undulating, and occasionally fringed with pseudomycelium. Acetic acid is produced.

Dalmau plate cultures on potato-, rice- and morphology agars: After 5 days at 25°C, pseudomycelium is usually abundantly produced under aerobic and anaerobic conditions. It consists of filamentous cells with or without blastospores, which may be arranged in chains or small somewhat branched verticils.

Formation of ascospores: Vegetative cells are directly transformed into asci, which produce one to four ascospores that are hat-shaped or spheroidal with tangential brims (Fig. 60). Ascospores are liberated soon after maturation and tend to agglutinate. Sporulation is usually sparse. As mating types could not be recovered from heat-treated ascosporulating material, the strains studied are presumed to be homothallic.

Sporulation was observed in cultures at room temperature after 7–15 days on YM agar supplemented with vitamins.

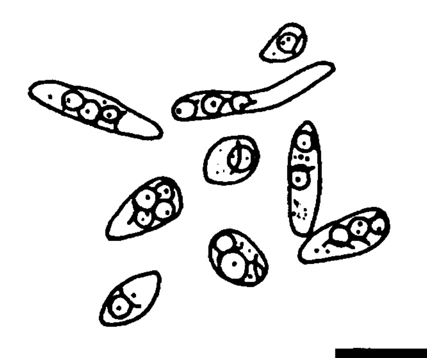


Fig. 60. *D. bruxellensis.* Asci with ascospores on YM agar + vitamins after 14 days at 25°C. Bar = 10 µm (from van der Walt 1970b).

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	–
Sucrose	v	Trehalose	v
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	v	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	v	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	v
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	–	0.1% Cycloheximide	v
0.01% Cycloheximide	+	Growth at 37°C	+

Co-Q: 9 (Yamada et al. 1980, Billon-Grand 1987).

Mol% G + C: 37.1–41.7, 21 strains, including CBS 72, CBS 73, CBS 74, CBS 75, CBS 78, CBS 1943, CBS 2976, CBS 5512, CBS 6955 (T_m: Smith et al. 1990b).

Origin of the strains studied: CBS 74, Belgium stout, Custers; CBS 72, lambic beer, Kufferath, type strain of *B. bruxellensis*; CBS 73, grape must, Krumbholz, type strain of *M. intermedia;* CBS 75, lambic beer, Custers, type strain of *B. lambicus*; CBS 78, beer, Skandinavik Bryggeri Laboratory, Copenhagen, type strain of *B. bruxellensis* var. *nonmembranaefaciens*; CBS 1943, sour wine, André, authentic strain of *B. bruxellensis* var. *vini*; CBS 2796, sparkling wine, Peynaud, type strain of *B. schanderlii*; CBS 4914, tea-beer, van der Walt, type strain of *D. intermedia*; CBS 5206, grape must, van der Walt; CBS 5512, equipment Bantu-beer brewery, van der Walt, type strain of *B. custersii*; CBS 6055, dry ginger ale, Ahearn, type strain of *B. abstinens;* CBS 8027, Coca Cola, Put; and 18 additional strains isolated from stout (2), wine (12), equipment beer brewery (1), porter (1), secondary fermentation (1), sherry (1).

Type strain: CBS 74 (ATCC 36234) isolated by Custers.

Comments: van der Walt (1964) separated *D. bruxellensis* and *D. intermedia* on the basis of morphological and physiological differences. However, Smith et al. (1990b) demonstrated by DNA complementarity and electrophoretic comparisons of enzymes, the conspecificity of *D. intermedia* with *D. bruxellensis*, the teleomorph–anamorph relationship of *D. bruxellensis* with *B. bruxellensis,* and the synonymy of *B. abstinens, B. custersii, B. intermedius* and *B. lambicus* with *D. bruxellensis.*

Comments on the genus

Until 1984, *Dekkera* was comprised of two species, *D. bruxellensis* and *D. intermedia* (van der Walt 1984a). Smith and van Grinsven (1984) described *D. anomala*, which they considered to be the teleomorph of *B. anomalus* on the basis of morphological and physiological criteria. These authors also observed the ascospores of this taxon by transmission electron microscopy. Subsequently, five more *Dekkera* species were described to represent the teleomorphs of five known *Brettanomyces* species (Lee and Jong 1985, 1986a,b, Jong and Lee 1986). From comparisons of isozyme similarities and DNA homologies, Smith et al. (1990b) accepted only two *Dekkera* species, viz. *D. anomala* and *D. bruxellensis* with *D. intermedia* as its synonym. Smith et al. (1990b) considered the reported ascospores (Lee and Jong 1985, 1986a,b, Jong and Lee 1986) as artifacts and did not accept *D. custersianus* and *D. naardenensis*. In addition, *D. abstinens* and *D. lambica* were placed in synonymy with *D. bruxellensis*, and *D. claussenii* was considered a synonym of *D. anomala*. This species concept was supported by the results of restriction analysis of genes coding for rRNA by Molina et al. (1993).

Various mtDNA-based analyses have been performed. McArthur and Clark-Walker (1983) examined nine *Dekkera/Brettanomyces* species and reduced the number to six on the basis of similarities in restriction enzyme digestion patterns, i.e., *D. bruxellensis (B. lambicus), D. intermedia, B. custersii (B. abstinens), B. anomalus (B. claussenii), B. custersianus* and *B. naardenensis*. In subsequent studies in which *Eeniella nana* was included, both the gene order (Hoeben and Clark-Walker 1986) and the nucleotide sequence of the mitochondrial-encoded cytochrome oxidase subunit gene (COX2) were determined (Clark-Walker et al. 1987, Hoeben et al. 1993). From these data, the preceding authors recognized five species, *D. bruxellensis (D. intermedia), B. custersii, B. anomalus, B. custersianus* and *B. naardenensis*. They also concluded that either *E. nana* should be included in *Brettanomyces* or *B. naardenensis* should be excluded from this genus.

From comparisons of partial sequences of 26S rDNA, Boekhout et al. (1994) accepted *D. bruxellensis, D. anomala, B. naardenensis* and *B. custersianus*, as well as *E. nana,* in *Brettanomyces*. The taxonomic position of *B. custersii*, considered conspecific with *B. bruxellensis* by Smith et al. (1990b), remained unsettled. Yamada et al. (1994d) studied the phylogeny of the four *Dekkera* species based on partial sequences of 18S and 26S rRNAs. These authors concluded that *D. custersiana* was phylogenetically distant from *D. bruxellensis (B. bruxellensis), D. anomala (B. anomalus)* and *D. naardenensis*. However, they postponed introduction of a new genus for this species until ascospore formation is confirmed.

28. *Dipodascopsis* Batra & P. Millner

M.Th. Smith and G.S. de Hoog

Diagnosis of the genus

Colonies are whitish, moist, somewhat slimy; true hyphae are present; arthroconidia are absent; multilateral budding cells are sometimes present. Asci are acicular or cylindrical, and formed laterally on hyphae after fusion of gametangia. Asci have persistent walls, and open by rupture at the apex. Ascospores are 32–128 per ascus, hyaline, ellipsoidal to reniform, and with a smooth wall and without a slime sheath.

Fermentation is absent. Extracellular starch is produced. Diazonium blue B reaction is negative. Coenzyme Q-9 system is present. Cell walls are multilamellar. Septa have narrow pores.

Type species

Dipodascopsis uninucleata Batra & P. Millner

Species accepted

1. *Dipodascopsis tothii* (Zsolt) Batra & P. Millner (1978)
2. *Dipodascopsis uninucleata* (Biggs) Batra & P. Millner (1978)
   a. *Dipodascopsis uninucleata* (Biggs) Batra & P. Millner var. *uninucleata* (1974)
   b. *Dipodascopsis uninucleata* var. *wickerhamii* Kreger-van Rij (1974)

Key to species

See Table 22.

1. a Cellobiose and lactose assimilated .... *D. tothii*: p. 178
   b Cellobiose and lactose are not assimilated → 2

2(1). a Sucrose and raffinose assimilated .... *D. uninucleata* var. *uninucleata*: p. 179
   b. Sucrose and raffinose are not assimilated .... *D. uninucleata* var. *wickerhamii*: p. 179

Table 22
Key characters in the genus *Dipodascopsis*

Species	Assimilation[a]										Ascus[a]
	Arb	Rha	Suc	Cel	Sal	Mel	Lac	Raf	Inu	37°	
Dipodascopsis tothii	+	−	+	+	+	−	+	+	+	−	bipod
D. uninucleata var. *uninucleata*	−	−	+	−	−	+	−	+	+	+	inter
D. uninucleata var. *wickerhamii*	−	+	−	−	−	+	−	−	−	+	inter

[a] Abbreviations: Arb, arbutin; Rha, L-rhamnose; Suc, sucrose; Cel, cellobiose; Sal, salicin; Mel, melibiose; Lac, lactose; Raf, raffinose; Inu, inulin; bipod, bipodal; inter, intercalary.

Systematic discussion of the species

28.1. *Dipodascopsis tothii* (Zsolt) Batra & P. Millner (Batra 1978)

Synonym:
Dipodascus tothii Zsolt (1963)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 4 mm in diameter, cream-colored, moist, and slimy. Budding cells are absent. Hyphae are firm with inflated cells and strongly amyloid. Arthroconidia are absent.

Formation of ascospores: Gametangia are formed laterally on hyphae as curved branches; asci arise after fusion of a terminal cell with its penultimate cell. Asci are acicular, 50–100 µm long, and contain 30–120 ascospores. Asci open by rupturing at the apex. Ascospores are subhyaline to hyaline, broadly ellipsoidal, occasionally bean-shaped, 1.0×(1.5–2.0) µm, and without a slime sheath (Fig. 61). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	−
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	−
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	−
Lactose	+	D-Mannitol	+
Melibiose	−	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	v
Soluble starch	+	DL-Lactate	−
D-Xylose	+	Succinate	w
L-Arabinose	+	Citrate	−
D-Arabinose	+	Inositol	+
D-Ribose	−	Hexadecane	n
L-Rhamnose	−	Nitrate	−
D-Glucosamine	−	Vitamin-free	−

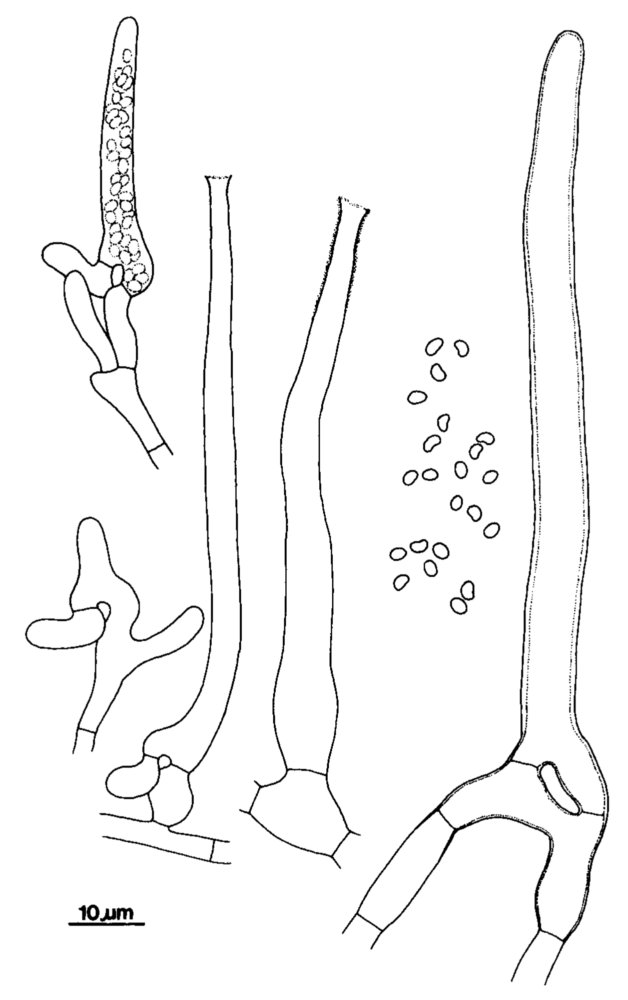


Fig. 61. *D. tothii*, CBS 759.85. Asci in various stages of maturation; liberated ascospores. MEA, 2 weeks, 20°C.

Additional assimilation tests and other growth characteristics:

Xylitol	+	50% Glucose	+
L-Arabinitol	+	60% Glucose	–
Arbutin	+	0.1% Cycloheximide	+
Cadaverine	+	Glucono-δ-lactone	–
L-Lysine	+	Acetic acid production	–
Ethylamine	w	Growth at 37°C	–

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: CBS 759.85 (NRRL Y-12690), from a cutting of beech (*Fagus sylvatica*), Hungary, Zsolt.

Type strain: CBS 759.85, from wood.

Comments: In contrast to *D. uninucleata*, gametangia of *D. tothii* extend from the hyphae which results in the formation of bipodal asci similar to those known in *Dipodascus*. The amyloid cell walls, absence of arthroconidia and extremely small ascospores lacking slime sheaths justify the classification of *D. tothii* in *Dipodascopsis*. Cells are uninucleate (Zsolt 1963). Ascospores become spheroidal after maturation, but further inflation and occasional budding, as seen in *D. uninucleata*, remain absent.

Ultrastructure of ascosporogenesis was studied extensively by Curry (1985). He stressed the presence of vesicles surrounding each spore-initial which is in contrast to the ascus vesicles known in the Euascomycetes. Van der Walt et al. (1990a) demonstrated the presence of the siderophores ferrichrome-C and a hitherto unidentified ferrichrome in *D. tothii*.

28.2. *Dipodascopsis uninucleata* (Biggs) Batra & P. Millner (Batra 1978)

This species has two varieties:

Dipodascopsis uninucleata (Biggs) Batra & P. Millner var. _uninucleata_ (1974)
Synonym:
Dipodascus uninucleatus Biggs (1937)

Dipodascopsis uninucleata var. _wickerhamii_ Kreger-van Rij (1974)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 4 mm in diameter, cream-colored, moist and slimy. Budding cells are sparse, spheroidal and thick-walled. Newly formed cells break through the parent cell wall and develop in more or less sympodial order. Hyphae are firm, with inflated cells, and produce abundant extracellular amyloid material. Arthroconidia are absent.

Formation of ascospores: Gametangia are undifferentiated adjacent cells or hyphal tips which fuse. Asci are produced laterally on the hyphae. Asci are acicular, 100–130 µm long and contain 30–120 ascospores. Asci open by rupturing at the apex. Ascospores are hyaline or subhyaline, ellipsoidal to bean-shaped, (0.8–1.2)×(2.0–2.8) µm, and without a slime sheath (Fig. 62). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	+	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	+/w	Inositol	+
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Xylitol	+	50% Glucose	+
L-Arabinitol	+	60% Glucose	–
Arbutin	–	0.1% Cycloheximide	+
Cadaverine	+	Glucono-δ-lactone	+
L-Lysine	+	Acetic acid production	–
Ethylamine	w	Growth at 37°C	+

Co-Q: Not determined.
Mol% G+C: Not determined.

Supplementary description of *D. uninucleata* var. *wickerhamii*: The variety *wickerhamii* does not assimilate sucrose, raffinose and inulin but assimilates L-rhamnose.

Origin of the strains studied belonging to the variety *uninucleata*: CBS 190.37, from dead pupa of a fruit fly (*Drosophila melanogaster*), Canada, Biggs; CBS 740.74 (NRRL Y-1268), from fruit fly, Mrak.

Type strain: CBS 190.37, from pupa.

Origin of the strain studied belonging to the variety *wickerhamii*: CBS 741.74 (NRRL Y-2181), from fruit fly (*Drosophila melanogaster*), Phaff.

Type strain: CBS 741.74, from fruit fly.

Comments: The most recent description was that given by Kreger-van Rij (1974). She attributed the binomial to Batra & Millner in an article cited as "in press" but actually it was published four years later. Consequently, the validation of *Dipodascopsis* should probably be attributed to Kreger-van Rij (1974). Kreger-van Rij (1974) noted that assimilation of soluble starch was variable, while D-mannitol and D-glucitol growth reactions were recorded as negative.

Budding cells are sparse. They arise from ascospores and are often found within the ascus. Liberated spores become thick-walled, occasionally form a few yeastlike cells but more often germinate with hyphae. Ascospores and hyphal cells are uninucleate (Biggs 1937). Hyphae bear hairy capsules and have multilamellar cell walls; septa are perforated by narrow, central canals with rounded edges (Kreger-van Rij and Veenhuis 1974). Van der Walt et al. (1990a) demonstrated the presence of ferrichrome as the main type of hydroxamate siderophores.

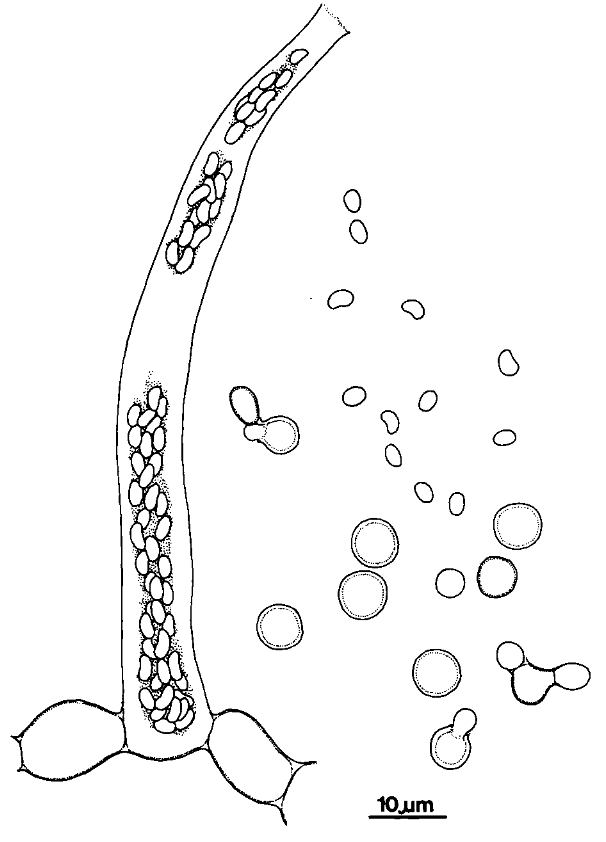


Fig. 62. *D. uninucleata* var. *uninucleata*, CBS 190.37. Ascus containing ascospores; liberated ascospores swell, become thick-walled prior to germination and occasionally form yeastlike cells. MEA, 2 weeks.

Comments on the genus

The taxonomic position of the genus *Dipodascopsis* is close to *Lipomyces* as discussed by Cottrell and Kock (1989), who used the presence of linolenic acid as a marker. Mitochondrial DNAs of *D. uninucleata* and other members of the Lipomycetaceae were compared by Lodolo et al. (1990); restriction patterns were found different in each genus.

29. *Dipodascus* de Lagerheim

G.S. de Hoog, M.Th. Smith and E. Guého

Diagnosis of the genus

Colonies are white or cream-colored, farinose or hairy, and usually dry; hyphae are hyaline, mostly disarticulating into rectangular arthroconidia (anamorph genus *Geotrichum*). Asci are acicular, cylindrical, ellipsoidal or subglobose, formed after fusion of gametangia located laterally on hyphae. Septa have micropores.

Asci have persistent walls and open by rupture at the apex. Ascospores are 4–128 per ascus, hyaline, ellipsoidal, with smooth walls and surrounded by regular slime sheaths.

Fermentation is mostly absent. Extracellular starch is not produced. Diazonium blue B reaction is negative.

Type species

Dipodascus albidus de Lagerheim

Species accepted

1. *Dipodascus aggregatus* Francke-Grosmann (1952)
2. *Dipodascus albidus* de Lagerheim (1892)
3. *Dipodascus ambrosiae* de Hoog, M.Th. Smith & Guého (1986)
4. *Dipodascus armillariae* W. Gams (1983)
5. *Dipodascus australiensis* von Arx & Barker (1977)
6. *Dipodascus capitatus* de Hoog, M.Th. Smith & Guého (1986)
7. *Dipodascus geniculatus* de Hoog, M.Th. Smith & Guého (1986)
8. *Dipodascus ingens* Rodrigues de Miranda ex de Hoog, M.Th. Smith & Guého (1997)
9. *Dipodascus macrosporus* Madelin & Feest (1982)
10. *Dipodascus magnusii* (Ludwig) von Arx (1977)
11. *Dipodascus ovetensis* (Peláez & C. Ramírez) von Arx (1977)
12. *Dipodascus spicifer* de Hoog, M.Th. Smith & Guého (1986)
13. *Dipodascus tetrasperma* (Macy & M.W. Miller) von Arx (1977)

Key to species

See Table 23.

Morphological key (also see under *Geotrichum*):

1.	a	Asci acicular or long-cylindrical, with a narrow apex → 2		
	b	Asci usually globose or ellipsoidal; when cylindrical, with a broadly rounded apex → 3		
2(1).	a	Asci and ascospores cylindrical	*D. macrosporus*:	p. 189
	b	Asci subulate; ascospores ellipsoidal	*D. albidus*:	p. 183
3(1).	a	Asci 1–4-spored → 7		
	b	Asci containing more than 4 spores → 4		
4(3).	a	Asci cylindrical, up to 120 µm long, in rather dense groups, containing up to 30 ascospores; insect symbiont	*D. aggregatus*:	p. 182
	b	No combination of the above characters → 5		
5(4).	a	Ascospores (2.8–3.2)×(3–4) µm; asci asymmetrically bipodal, somewhat tapering towards the tip	*D. geniculatus*:	p. 187
	b	Ascospores larger; asci cylindrical to ellipsoidal → 6		
6(5).	a	Asci mostly in groups, broadly ellipsoidal, mostly present in culture; on rotting parts of tropical or subtropical succulents	*D. australiensis*:	p. 185
	b	Asci solitary, rather irregular in shape, not formed in culture; on carpophores of *Armillaria* in temperate zone	*D. armillariae*:	p. 184
7(3).	a	Asci borne on erect or suberect hyphae, anisogamous; ascospores (5.0–6.5)×(8.5–11.0) µm	*D. magnusii*:	p. 189
	b	Asci borne on undifferentiated hyphae, isogamous; ascospores smaller → 8		
8(7).	a	Asci usually longer than wide → 9		
	b	Asci appressed, usually shorter than wide; hyphae straight and stiff, 7–9 µm wide, with acuminate apices	*D. tetrasperma*:	p. 192
9(8).	a	Sympodial rachides abundant → 12		
	b	Sympodial rachides absent or scarce → 10		
10(9).	a	Initial growth with pseudomycelium → 11		
	b	Initial growth with true hyphae	*D. ambrosiae*:	p. 184
11(10).	a	Thallus entirely pseudomycelial	*D. ingens*:	p. 188
	b	Thallus initially pseudomycelial, changing into true hyphae	*D. ovetensis*:	p. 190
12(9).	a	Branching regular, often verticillate; rachides straight; on warm-blooded animals	*D. capitatus*:	p. 186
	b	Branching rather irregular; rachides flexuose; on rotting parts of tropical or subtropical succulents	*D. spicifer*:	p. 191

Physiological and morphological key, see under *Geotrichum*

Table 23
Key characters of species in the genus *Dipodascus*

Species	Assimilation							Hyphae[a]	Asci[b]	Rachides[c]	Mating system
	Maltose	Cellobiose	Raffinose	D-Xylose	Glucitol	Salicin	Citrate				
Dipodascus aggregatus	–	–	–	+	+	–	+	+	Cylindrical	–	Homothallic
D. albidus	–	–	–	+	+	–	–	+	Acicular	–	Homothallic
D. ambrosiae	–	–	–	–	+	–	–	+	Ellipsoidal	–	Homothallic
D. armillariae	–	+	–	+	v	–	v	+	Cylindrical	–	?
D. australiensis	–	–	–	+	+	–	–	+	Cylindrical	v	Homothallic
D. capitatus	–	–	–	–	–	–	v	+	Ellipsoidal	+	Heterothallic
D. geniculatus	+	–	–	+	+	–	+	+	Cylindrical	–	Homothallic
D. ingens	–	–	–	–	–	–	–	–	Ellipsoidal	–	Heterothallic
D. macrosporus	–	+	–	+	+	–	+	+	Tubular	–	Homothallic
D. magnusii	–	–	+	–	+	–	–	+	Ellipsoidal	–	Homothallic
D. ovetensis	–	–	–	–	–	–	–	+	Ellipsoidal	–	Homothallic
D. spicifer	–	+	–	+	–	+	+	+	Ellipsoidal	+	Homothallic
D. tetrasperma	–	–	–	–	+	–	+	+	Ellipsoidal	–	Homothallic

[a] Presence of hyphae.
[b] Shape of asci.
[c] Sympodial rachides present.

Systematic discussion of the species

29.1. *Dipodascus aggregatus* Francke-Grosmann (1952)

Synonym:
Dipodascus albidus de Lagerheim var. *minor* Korf (1957)

Growth on 4% malt extract/0.5% yeast extract agar (MEYA): After 10 days at 20–22°C, colonies are 25 mm in diameter, glassy whitish, with a hispid center and smooth margin. Expanding hyphae are parallel, flexuose, 6–8 μm wide, with some basitonous branching; lateral branches are 3–5 μm wide, disarticulating into rectangular arthroconidia which are (2.6–5.0)×(6–10) μm, and that soon intermingle with larger conidia from main branches.

Formation of ascospores: Hyphae locally become densely septate and form clusters of isogamic gametangia. Asci are aggregated in small groups, cylindrical with rounded apex, (7–10)×(40–120) μm, mostly with 10–30 ascospores which are liberated by apical rupture of the ascus. Ascospores are subhyaline, broadly ellipsoidal, (3.0–4.5)×(3.8–6.0) μm, with a slime sheath 1.2–1.6 μm thick (Fig. 63). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: Not determined.

Mol% G+C: 45.4–45.8, CBS 152.57, CBS 284.86 (BD: Guého et al. 1985).

Origin of the strains studied: CBS 175.53, from pupal galleries of bark-beetle *Ips acuminatus* in pine *(Pinus*

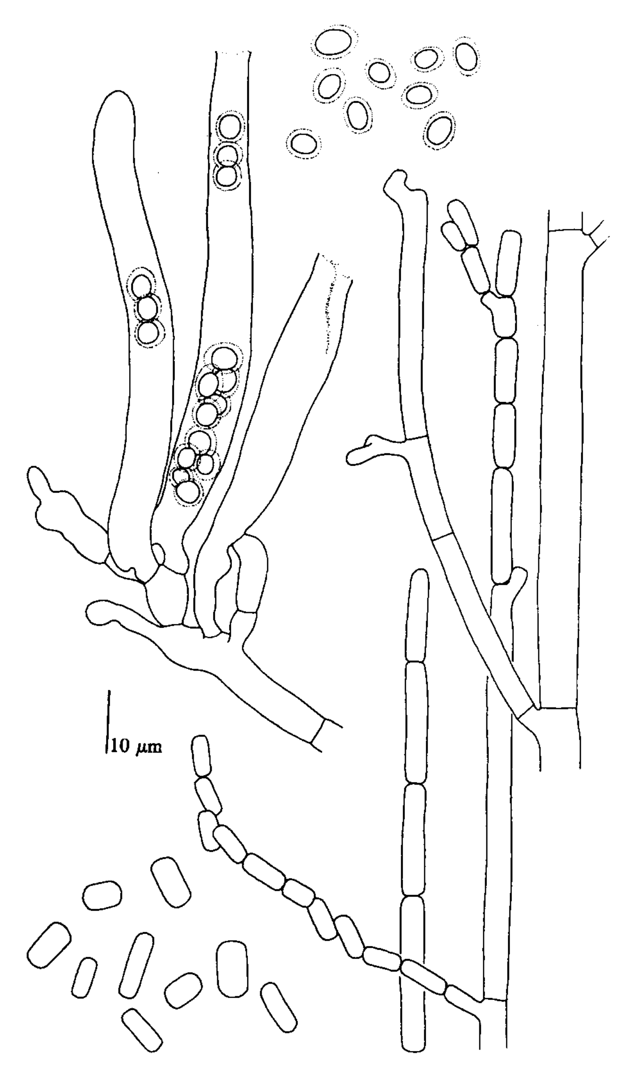


Fig. 63. *D. aggregatus*, CBS 175.53. Asci with ascospores; anamorph composed of hyphae disarticulating into arthroconidia. MEYA, 22°C.

sylvestris), Francke-Grosmann, Sweden; from frass of *Ips* in pine (*Pinus* sp.) roots (1); from slime flux of pine (*Pinus* sp.) attacked by *Ips* (1) or *Dendroctonus* (1); from unknown source (2).

Lectotype strain: CBS 175.53.

Comments: The species is obligately associated with bark beetles behind the bark of *Pinus* (Batra 1967). *D. aggregatus* is recognized by its broadly cylindrical asci with dome-shaped apices. Asci are easily produced on most routine media. The species was recently redescribed by Tsuneda (1987, 1989).

29.2. *Dipodascus albidus* de Lagerheim (1892)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 30 mm in diameter, white, farinose, and finally hispid. The reverse is whitish. Expanding hyphae are parallel, 6–8 μm wide, poorly branched, with little differentiation of main branches, and soon disarticulate into rectangular arthroconidia.

Formation of ascospores: Gametangia are mostly identical, and mostly formed on opposite sides of a septum. Asci are slightly swollen at the base, then tapering, acicular, 180–380 μm long, with narrow apical opening to release the spores. Ascospores are up to 128 per ascus, hyaline or subhyaline, broadly ellipsoidal, (1.2–1.8)×(1.8–3.2) μm, and with a slime sheath 0.5–1.0 μm thick (Fig. 64). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

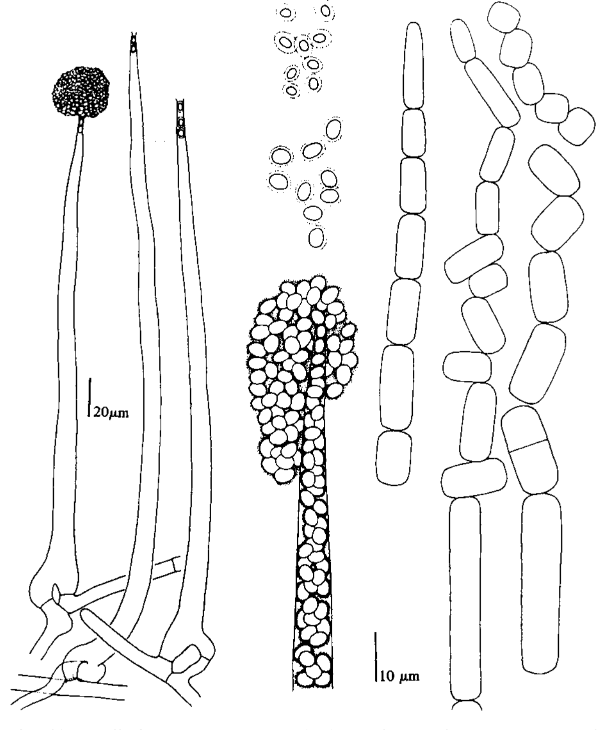


Fig. 64. *D. albidus*, CBS 766.85. Acicular asci liberating ascospores and hyphae disarticulating into arthroconida. MEYA, 22°C.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: Not determined.
Mol% G+C: 41.9, CBS 766.85 (T_m: de Hoog et al. 1986).

Origin of the strain studied: CBS 766.85 (IFO 1984), from exudate of a broadleaf tree, Nakase, Japan.

Neotype herbarium specimen: CUP 20140, Juel.

Comments: This very characteristic species is restricted to exudates of deciduous trees. There is some morphological resemblance to species of *Dipodascopsis,* but these have curved ascospores and produce extracellular starch.

29.3. *Dipodascus ambrosiae* de Hoog, M.Th. Smith & Guého (1986)

Growth on 4% malt extract/0.5% yeast extract agar: Colonies after 10 days are 13 mm in diameter, whitish, buttery, somewhat moist, smooth, with finely lobed margins. Hyphae arise bilaterally from ends of inflated cells, straight, without differentiation of main and lateral branches, mostly 2.5–3.5 μm wide, disarticulating into rectangular cells up to 20 μm long; liberated conidia often percurrently proliferate at their ends.

Formation of ascospores: Gametangia are formed on different hyphae or at opposite sides of hyphal septa. Asci are broadly ellipsoidal, (6–7)×(10–13) μm, and contain 2–4 ascospores. Ascospores are hyaline or subhyaline, ellipsoidal, (3–4)×(4.5–5.5) μm, and with a slimy sheath 1–2 μm thick (Fig. 65). The species is homothallic.

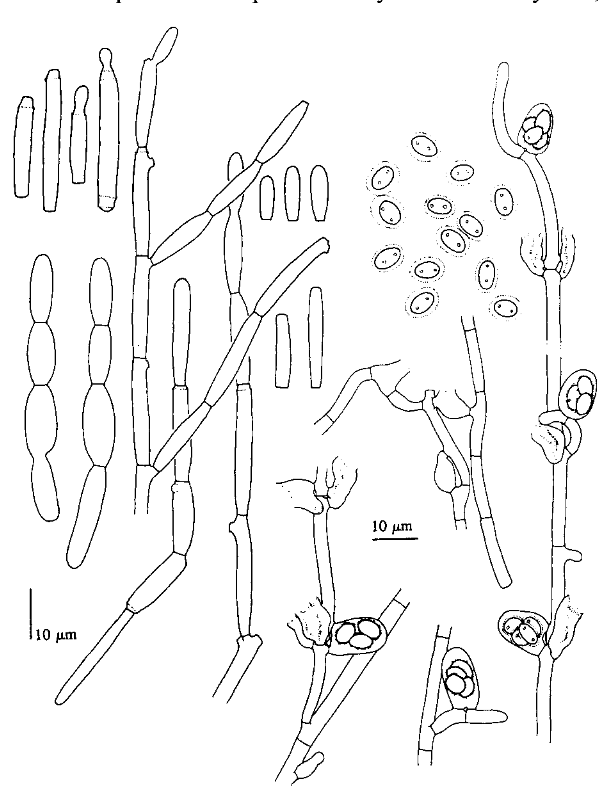


Fig. 65. *D. ambrosiae,* CBS 749.85. Early development of hyphae and mature hyphae with terminal and intercalary arthroconidia. Some asci have liberated their ascospores. MEYA, 22°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: Not determined.
Mol% G+C: 45.2, CBS 749.85 (T_m: de Hoog et al. 1986).

Origin of the strain studied: CBS 749.85 (BPI 3528), from insect tunnel, Batra, California.

Type strain: CBS 749.85.

Comments: The species is only known from the type locality. It might be one of the bark insect-associated species, but is clearly distinguished from *D. aggregatus* by small asci and a different hyphal structure. In these respects it is much more similar to *D. ovetensis,* but has considerably smaller asci and ascospores.

29.4. *Dipodascus armillariae* W. Gams (1983)

Anamorph: *Geotrichum decipiens* (L.R. Tulasne & C. Tulasne) W. Gams

Synonyms:
Hypomyces decipiens L.R. Tulasne & C. Tulasne (1865)
Geotrichum decipiens (L.R. Tulasne & C. Tulasne) W. Gams (1983)
Geotrichum armillariae von Arx (1977a)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 20–30 mm in diameter, white, dry, and centrally powdery to velvety. Expanding hyphae are loose, with irregular basitonous branching at acute angles; hyphae are 3.5–5.5 μm wide, with little differentiation between main and lateral branches, and soon disarticulate into rectangular arthroconidia (3.5–4.2)×(7–18) μm. Pale brown, thick-walled, subglobose chlamydospores measuring (12–16)×(13–17) μm are often present.

Formation of ascospores: Gametangia are formed only on the natural substrate, and located on opposite sides of

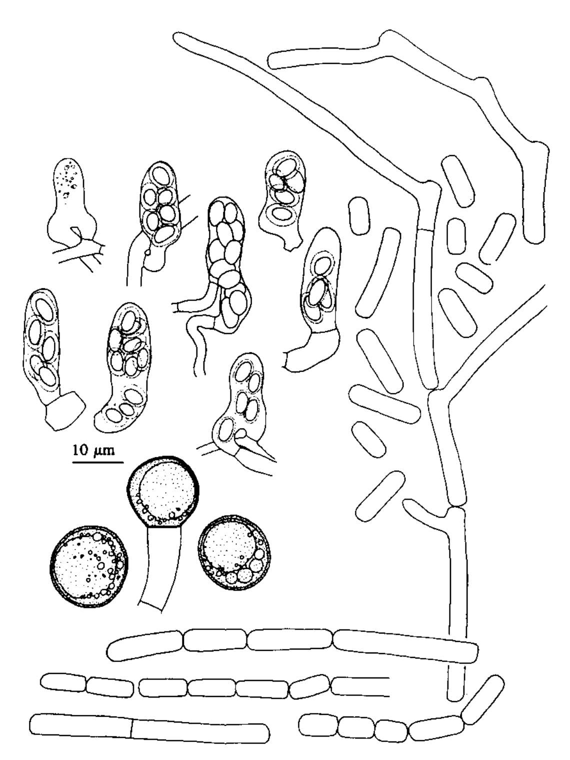


Fig. 66. *D. armillariae.* Asci on natural substrate, CBS-H 3348; hyphae disarticulate into arthroconidia, CBS 540.76, MEYA, 22°C; chlamydospores, CBS 817.71, MEYA, 22°C.

hyphal septa. Asci are broadly ellipsoidal to cylindrical, (7–10)×(17–28) μm, and contain 4–12 ascospores. Ascospores are ellipsoidal, (2.5–4.0)×(4–6) μm, with a slime sheath (Fig. 66).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	–	Ethanol	v
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: Not determined.

Mol% G+C: 42.2–42.3, CBS 834.71, CBS 540.76 (BD: Guého et al. 1985).

Origin of the strains studied: CBS 817.71 (ATCC 36758), on gills of mushroom (*Armillaria* sp.), van der Aa, Netherlands; 9 additional strains from gills of the *Armillaria mellea* aggregate.

Type strain: CBS 817.71.

Comments: The species is thus far only known from carpophores of *Armillaria* species in the Northern hemisphere. Asci are only produced on the natural substrate during a short period in October. Single-spore isolations invariably gave the *Geotrichum* anamorph with characteristic chlamydospores (de Hoog et al. 1986).

The species has often been confused with *Endomyces decipiens* Reess, but that species has hat-shaped ascospores. Nomenclatural separation of the two unrelated taxa was discussed by Gams (1983).

29.5. *Dipodascus australiensis* von Arx & Barker (von Arx 1977a)

Growth on 4% malt extract/0.5% yeast extract agar: Colonies, after 10 days at 20–22°C, are 30–60 mm in diameter, white, evenly felty to fluffy, and with a straight margin. Expanding hyphae locally with dichotomous branching, main branches 4–7 μm wide, basitonously branched at acute angles, lateral branches 3–4 μm wide, and soon disarticulate into rectangular arthroconidia (3.5–5.5)×(6–12) μm.

Formation of ascospores: Equal gametangia are formed on opposite sides of septa; asci are subglobose to broadly clavate, (6–11)×(15–45) μm, with mostly 4–12 ascospores. Ascospores are subhyaline, ellipsoidal, often unilaterally flattened, (2.5–4.0)×(4–6) μm, and with a slime sheath 1.2–1.6 μm thick (Fig. 67). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

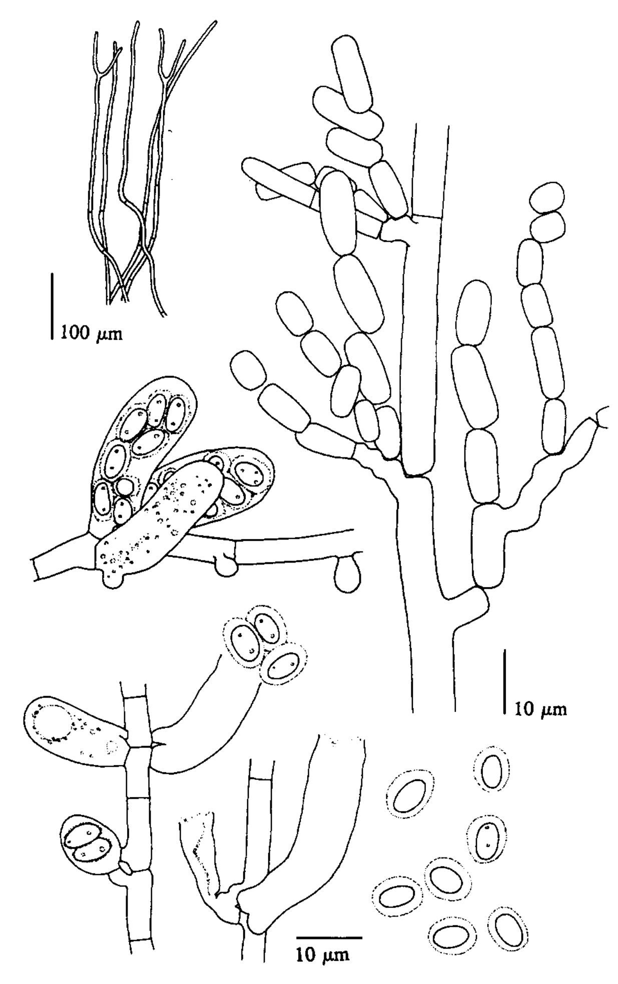


Fig. 67. *D. australiensis.* Branching pattern of expanding hyphae with hyphae disarticulating into arthroconidia, CBS 666.79; asci liberating ascospores, CBS 666.79, MEYA, 22°C.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	+
Arbutin	–		

Co-Q: Not determined.

Mol% G + C: 39.9–40.2, CBS 625.74, CBS 666.79 (T_m: de Hoog et al. 1986).

Origin of the strains studied: CBS 625.74, from rotting cladodes of cactus *Opuntia inermis,* Barker, Australia; from necrosis in *Opuntia* (2); from *Euphorbia* (1).

Type strain: CBS 625.74.

Comments: The species is restricted to rotten fruits and leaves of succulents in desert areas. Asci are easily produced on routine laboratory media. They are sometimes formed in small clusters at hyphal septa, and then there is much similarity to poorly developed cultures of *D. aggregatus*. However, *D. australiensis* has local, dichotomously branched, expanding hyphae and much basitonous branching, leading to geniculate side branches after hyphae have disarticulated. In addition, *D. australiensis* is unable to assimilate citrate, but does grow at 37°C. The two species clearly differ in their ecological preferences.

29.6. *Dipodascus capitatus* de Hoog, M.Th. Smith & Guého (1986)

Anamorph: *Geotrichum capitatum* (Diddens & Lodder) von Arx

Synonyms:

Trichosporon capitatum Diddens & Lodder (1942)
Geotrichum capitatum (Diddens & Lodder) von Arx (1977a)
Ascotrichosporon capitatum (Diddens & Lodder) Kocková-Kratochvílová, Slávikovà, Zemek & Kuniak (1977b)
Blastoschizomyces capitatus (Diddens & Lodder) Salkin, Gordon, Samsonoff & Rieder (1985)
Sporotrichum spicatum Delitsch (1943)
Geotrichum linkii Vörös-Felkai (1961)
Blastoschizomyces pseudotrichosporon Salkin, Gordon, Samsonoff & Rieder (1985)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 15–20 mm in diameter, glassy, later funiculose with a smooth expanding zone. Expanding hyphae are branched at acute angles, often somewhat penicillate, main branches up to 7.0 μm wide, lateral branches 2.5–3.5 μm wide and terminate in cylindrical conidiogenous cells with long, cicatrized rachides 2.5 μm wide. Conidia are clavate, with a truncate base, (2.5–3.5) × (7–10) μm. Arthroconidia are also common.

Formation of ascospores: Gametangia are formed at opposite sides of septa. Asci are subspherical to broadly ellipsoidal, (7–9) × (7–11) μm. Ascospores are hyaline, ellipsoidal, (2.5–3.2) × (5.5–6.5) μm, and with a persistent slime sheath 1–2 μm thick (Fig. 68). The species is heterothallic.

Ascospores are observed on vitamin-enriched V8 medium. Sporulation is sparse.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

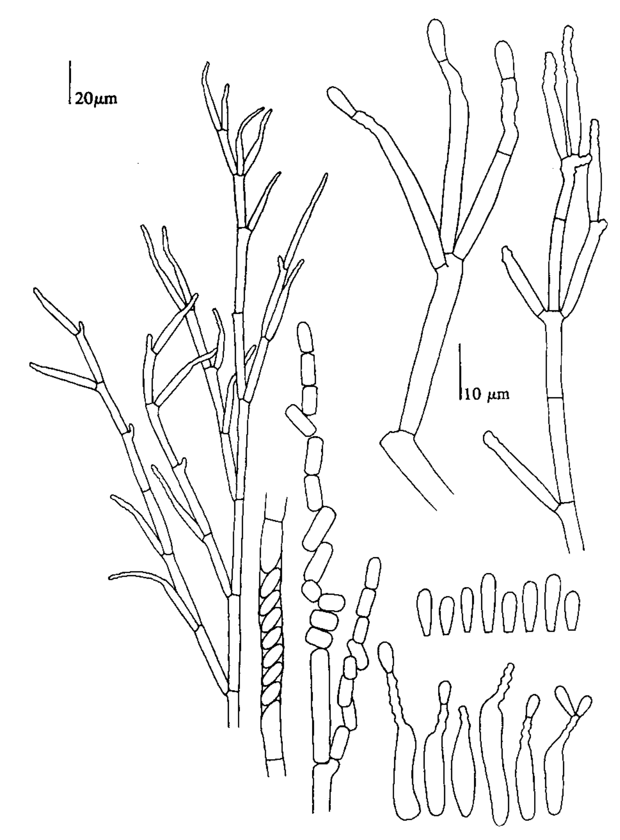


Fig. 68. *D. capitatus*. Conidial apparatus with sympodial rachides, sympodial conidia, arthroconidia and endoconidia, various cultures. MEYA, 22°C.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	+
Arbutin	–		

Co-Q: 9 (Yamada et al. 1982).

Mol% G+C: 32.3–36.3 (T_m: Guého et al. 1987a).

Origin of the strains studied: CBS 571.82 (CBS 2364, ATCC 10663), from wood pulp factory with an air-tight warming system, Rennerfeldt, lectotype strain of *Trichosporon capitatum*; CBS 578.82, from sputum, authentic strain of *Geotrichum linkii*, do Carmo-Sousa; CBS 207.83 (ATCC 46132), from sputum, type strain of *Blastoschizomyces pseudotrichosporon*, Salkin, U.S.A.; from wood pulp (2); sputum (5); bovine mastitic milk (1); yeast cake (1); oral infections (2); digestive tract of swine (1); blood (2).

Complementary mating types: CBS 197.35 and 580.82.

Type strain: CBS 197.35×580.82.

Comments: Asci with ascospores are found in very low abundance on nutritionally rich media. They need several weeks to fully mature. A characteristic feature of *D. capitatus* is the sympodially produced conidia which are cylindrical to clavate, with truncate base and rounded apex. Arthroconidia are rare in this species.

The species is associated with human lung disorders. Recently it is increasingly being found in blood of immunocompromised hosts, particularly in cases of leukemia.

29.7. *Dipodascus geniculatus* de Hoog, M.Th. Smith & Guého (1986)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 35 mm in diameter, white, flat, with fimbriate margin. Hyphae are stiff, branched at acute angles, 3–5 μm wide. Arthroconidia are absent.

Formation of ascospores: Gametangia are unequal and formed on opposite sides of septa; hyphae are often bent at the septa. Asci are broadly cylindrical to ellipsoidal, with a rounded apex and a strongly asymmetric base, (6–10)×(25–65) μm, and usually contain about 20 ascospores. Ascospores are subhyaline, broadly ellipsoidal, (2.8–3.2)×(3–4) μm, with a slime sheath 0.5 μm thick (Fig. 69). The species is homothallic.

Fermentation:

Glucose	w/–	Maltose	–
Galactose	–	Lactose	–
Sucrose	–	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: Not determined.

Mol% G+C: 43.2, CBS 184.80 (T_m: de Hoog et al. 1986).

Origin of the strain studied: CBS 184.80, from *Psidium guajava* pulp, Bhide, India.

Type strain: CBS 184.80.

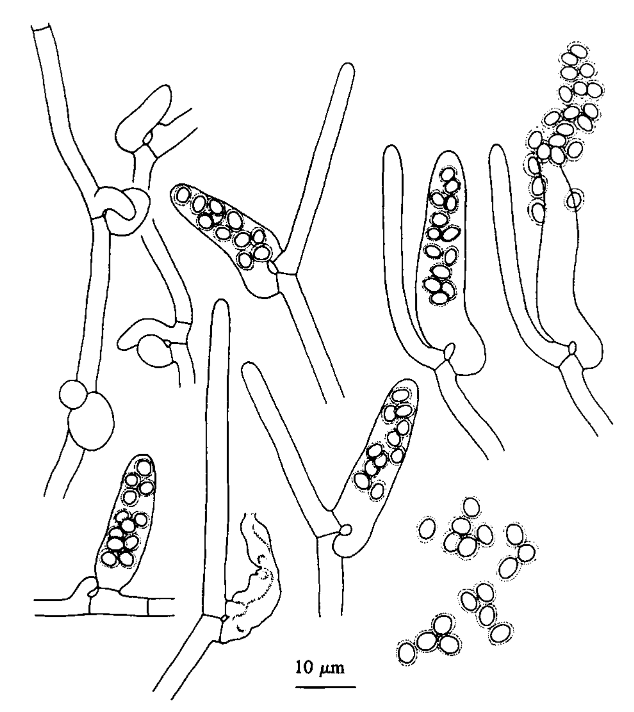


Fig. 69. *D. geniculatus*, CBS 184.80. Gametangia, asci and liberated ascospores. MEYA, 22°C.

Comments: The species, thus far only known from the type strain, can be recognized by absence of arthroconidia and by strongly asymmetric asci formed at the edges of kinks in supporting hyphae. Asci are produced in relative abundance on oatmeal agar, but only poorly on most other media. The species differs from *D. australiensis* by absence of arthroconidia, absence of growth at 37°C and by growth with maltose.

29.8. *Dipodascus ingens* Rodrigues de Miranda ex de Hoog, M.Th. Smith & Guého (1997a)

Anamorph: *Geotrichum ingens* (van der Walt & van Kerken) de Hoog, M.Th. Smith & Guého

Synonyms:
Candida ingens van der Walt & van Kerken (1961c)
Geotrichum ingens (van der Walt & van Kerken) de Hoog, M.Th. Smith & Guého (1997a)
Pichia humboldtii Rodrigues de Miranda & Török (1976)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 12 mm in diameter, glassy white, butyrous, smooth. The reverse is whitish. Hyphae are pseudomycelial, consisting of rounded-off cells of variable shape which are gradually narrower towards the distal end. Arthroconidia are cylindrical to ellipsoidal, and of variable size.

Formation of ascospores: Gametangia are mostly formed on different hyphae. Asci are broadly ellipsoidal, (10–17)×(12–20) μm, and contain 2–4 ascospores. Ascospores are hyaline or subhyaline, ellipsoidal, (3.0–4.5)×(5.5–7.0) μm, and with a slime sheath 1–3 μm thick (Fig. 70). The species is heterothallic.

Fermentation:

Glucose	w/–	Maltose	–
Galactose	–	Lactose	–
Sucrose	–	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Fig. 70. *D. ingens*. Pseudomycelial anamorph, CBS 7197, MEYA; asci with ascospores, CBS 7197×4827 and CBS 7197×4825, enriched V8, 22°C.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	v
Arbutin	–		

Co-Q: Not determined.

Mol% G+C: 39.5, CBS 4603 (T_m: de Hoog et al. 1986).

Origin of the strains studied: CBS 4603 (ATCC 60122, IFO 10057) type strain of *Candida ingens*; CBS 4827 and 2 additional strains, all from a wine cellar, van der Walt, South Africa; CBS 7197, Hoefer; from industrial sulphite waste (1); from phenolic waste water (1); from asphalt waste (1).

Complementary mating types: CBS 7197 and CBS 4827.

Type strain: CBS 7197×4827.

Comments: Judging from the list of sources of this fungus, there appears some preference for phenolic compounds. The species has been described as a supposedly homothallic species, *Pichia humboldtii* Rodrigues de Miranda & Török (1976), but with the discovery of heterothallism in this species, the earlier results were interpreted as endoconidia. The species is close to *D. ovetensis*; for differential diagnosis see under that species.

29.9. *Dipodascus macrosporus* Madelin & Feest (1982)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 5 mm in diameter, whitish, buttery, composed of minute, confluent satellite colonies, and become somewhat cerebriform. Expanding hyphae are 3.5–6.5 µm wide, branched at acute angles, and without differentiation of main branches.

Formation of ascospores: Terminal hyphal cells become cylindrical asci after fertilization at the base by adjacent hyphae. Asci are cylindrical, about 80 µm long, contain 10–30 uniseriate ascospores, and open by rupture at the apex. Ascospores are hyaline or subhyaline, cylindrical to ellipsoidal, (2.5–3.2)×(6–8) µm, and with a slime sheath 1.2–2.0 µm thick (Fig. 71). The species is homothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: Not determined.

Mol% G+C: 41.5, CBS 259.82 (T_m: de Hoog et al. 1986).

Origin of the strains studied: CBS 259.82 (IMI 263027) and an additional strain, both from the myxomycete *Badhamia utricularis*, Madelin and Feest, U.K.

Type strain: CBS 259.82.

Comments: This rare, homothallic species found on myxomycete thalli is easily recognizable by the absence of arthroconidia on agar media and the presence of terminal, cylindrical asci with subcylindrical ascospores. Arthroconidia may be formed in shaken liquid culture. Another mycoparasitic *Dipodascus* species is *D. armillariae*. This species occurs on Agaricales and forms its ellipsoidal asci only on natural substrates. Its anamorph is characterized by the presence of large, brown chlamydospores.

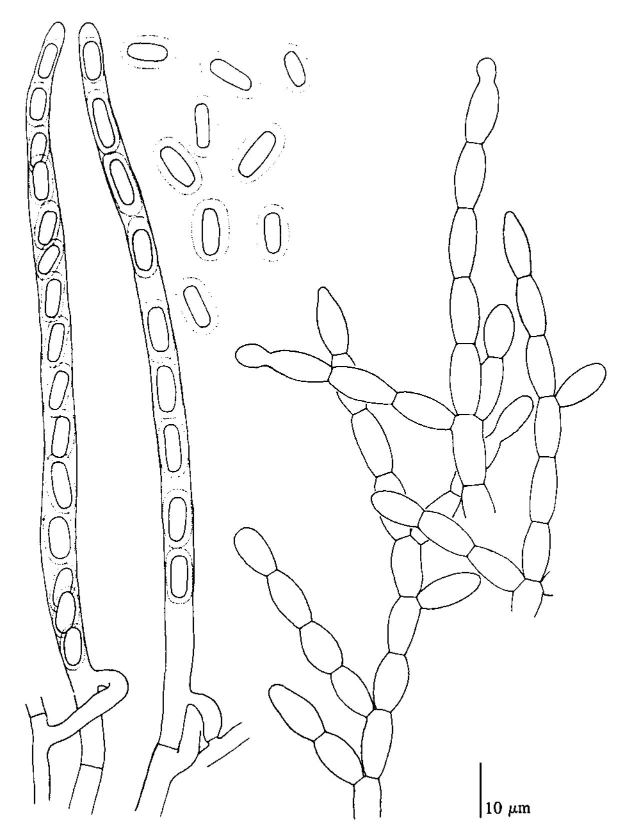


Fig. 71. *D. macrosporus*, CBS 259.82, MEYA, 22°C. Tubular asci with ascospores and pseudomycelial hyphae.

29.10. *Dipodascus magnusii* (Ludwig) von Arx (1977a)

Anamorph: *Geotrichum ludwigii* (Hansen) Fang, Cheng & Chu

Synonyms:

Endomyces magnusii Ludwig (1886)

Magnusiomyces ludwigii Zender (1925a)

Geotrichum ludwigii (Hansen) Fang, Cheng & Chu (1966)

Magnusiomyces magnusii (Ludwig) Zender (1925b)

Endyllium magnusii (Ludwig) Clements (Clements & Shear 1931)

Oospora magnusii Stautz (1931)
Oidium ludwigii Hansen (Holtz 1901)
Oospora ludwigii (Hansen) P. Saccardo & D. Saccardo (1906)
Geotrichum ludwigii (Hansen) Saëz (1968a)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 25 mm in diameter, glassy white, buttery; the margin is smooth and strongly lobed. Expanding hyphae are stiff; main branches are 7–12 µm wide, branched at acute angles in a penicillate manner, lateral branches are 4–7 µm wide, and soon disarticulate into rectangular arthroconidia (4–7)×(10–18) µm which often show some annellations at one or both ends.

Formation of ascospores: Gametangia are formed on the ends of side branches, an anisogamous, terminal cell being connected by a tube originating from a few cells further down. Asci are broadly ellipsoidal, (9–16)×(17–30) µm, containing 4 ascospores. Ascospores are subhyaline, ellipsoidal, (5.0–6.5)×(8.5–11.0) µm, and have a slime sheath 1.2–2.5 µm thick (Fig. 72). The species is homothallic.

Ascospores are observed on vitamin-enriched V8 medium.

Fermentation:

Glucose	+	Maltose	–
Galactose	+/w	Lactose	–
Sucrose	+	Raffinose	+/w

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: 9 (Yamada et al. 1973b).
Mol% G+C: 34, CBS 107.12 (T_m: Guého 1979), 37.8 (BD: Guého et al. 1985); 40.9, CBS 234.85 (T_m: de Hoog et al. 1986).

Origin of the strains studied: CBS 108.12, Lindner; CBS 151.30, type strain of *Oospora magnusii*, from exudate of oak (*Quercus* sp.), Stautz; CBS 234.85, from exudate of oak, Wright, Pennsylvania.

Possible type strain: CBS 108.12.

Comments: Asci are produced on enriched V8 juice agar, but often only in low abundance. CBS 107.12 and 108.12, which do not form asci in pure culture, are Ludwig's authentic strains, but unfortunately no exact collection data are available.

The species is probably restricted to the slime flux of oak. *D. magnusii* is remarkable for its anisogamous ascus production. Anascogenous cultures morphologically resemble *Geotrichum fragrans*, but do not produce the strong odor characteristic of the latter species.

The mol% G+C of 43.7 in CBS 234.85 was obtained after calculation of the derivative peak. With total DNA, 40.9 mol% was found (de Hoog et al. 1986). The marked spread of G+C values in *D. magnusii*, as well as in several other *Dipodascus* species is at least partly caused by considerable amounts of mtDNA or rDNA with a lower mol% G+C.

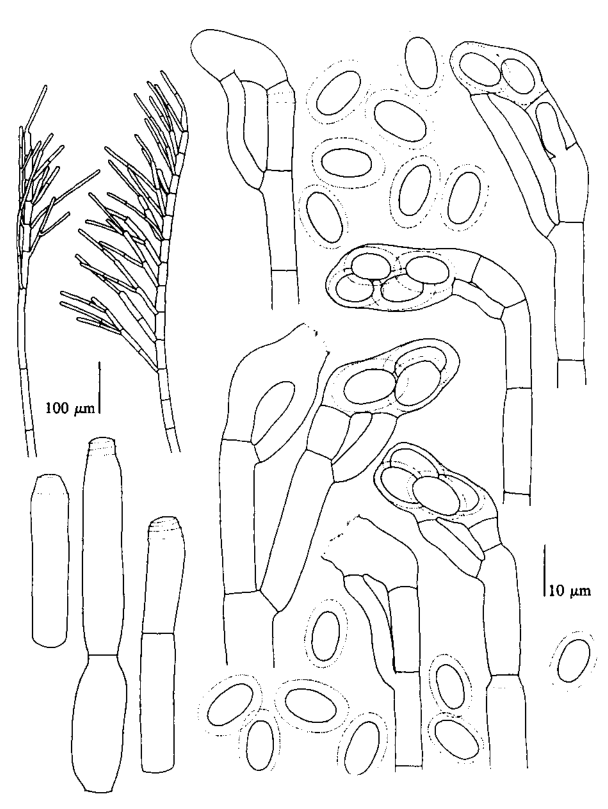


Fig. 72. *D. magnusii.* Branching pattern of expanding hyphae, arthroconidia with annellated zones, CBS 107.12, MEYA; anisogamous asci liberating ascospores, CBS 234.85, enriched V8, 22°C.

29.11. *Dipodascus ovetensis* (Peláez & C. Ramírez) von Arx (1977a)

Anamorph: *Geotrichum sericeum* (Stautz) de Hoog, M.Th. Smith & Guého

Synonyms:
Endomyces ovetensis Peláez & C. Ramírez (1956b)
Endomycopsis ovetensis (Peláez & C. Ramírez) Kreger-van Rij (1964a)
Zendera ovetensis (Peláez & C. Ramírez) Redhead & Malloch (1977)
Oospora sericea Stautz (1931)
Trichosporon sericeum (Stautz) Diddens & Lodder (1942)

Ascotrichosporon sericeum (Stautz) Kocková-Kratochvílová, Slávikova, Zemek & Kuniak (1977b)
Geotrichum sericeum (Stautz) de Hoog, M.Th. Smith & Guého (1986)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 15–25 mm in diameter, whitish, moist, buttery. Expanding hyphae are 4.5–6.0 µm wide, arising from a short series of inflated cells, little branched with narrower lateral branches 3.5–5.0 µm wide, and disarticulating into cylindrical arthroconidia about 10–30 µm length.

Formation of ascospores: Gametangia are mostly located on different hyphae. Asci are broadly ellipsoidal, contain 2–4 ascospores and rupture irregularly at maturity. Ascospores are hyaline or subhyaline, ellipsoidal, (3.0–4.5)×(5.5–7.0) µm, and with a slime sheath 1–3 µm thick (Fig. 73). The species is homothallic.

Ascospores are observed on vitamin-enriched V8 medium.

Fermentation:

Glucose	w/–	Maltose	–
Galactose	–	Lactose	–
Sucrose	–	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: 9 (Yamada et al. 1973b).

Mol% G + C: 51.2–51.5, CBS 192.55, CBS 634.85 (T_m: de Hoog et al. 1986).

Origin of the strains studied: CBS 192.55 (CBS 2508), from tannin concentrate, Ramírez; CBS 634.85 (CBS 2544), authentic strain of *Oospora sericea*, from slime flux of oak (*Quercus* sp.), Stautz, Germany; from slime flux of oak (3); from unknown source (1).

Type strain: CBS 192.55.

Comments: The species seems to be restricted to the slime fluxes of deciduous trees, *Quercus* in particular. The strain from tannin concentrate initially may have had a similar ecological niche.

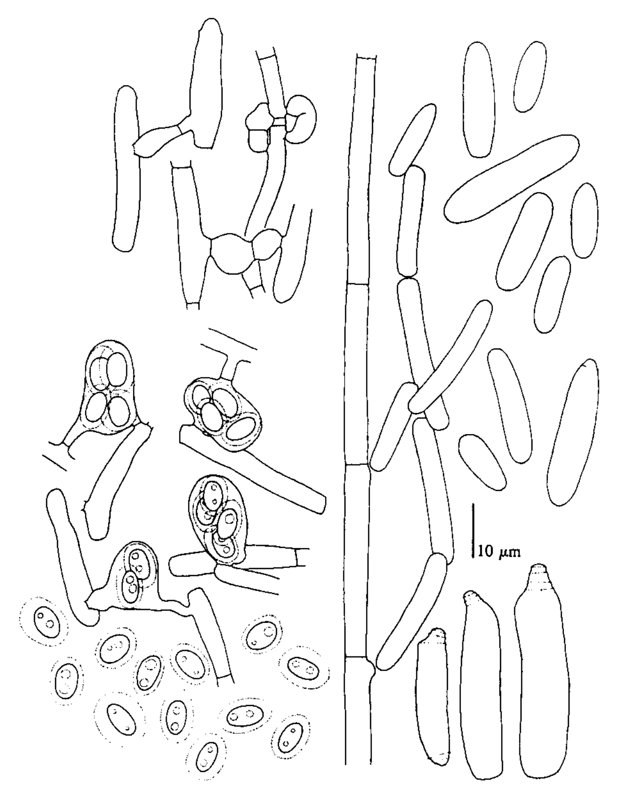


Fig. 73. *D. ovetensis.* Gametangia, mature asci and liberated ascospores, CBS 635.85, enriched V8; hyphae disarticulate into arthroconidia and conidia with annellated zones, CBS 192.55, MEYA, 22°C.

The species is close to *D. ingens*, but has true hyphae. In addition, larger conidia often show percurrent proliferation (de Hoog et al. 1986). The two species are physiologically identical but have remarkably different G + C percentages of their DNAs.

29.12. *Dipodascus spicifer* de Hoog, M.Th. Smith & Guého (1986)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 20 mm in diameter, whitish, with a regular, straight margin; the texture is soft and butyrous. Expanding hyphae are loose and branched at acute angles; hyphae are 2.5–5.0 µm wide, with little differentiation of main and lateral branches; locally they terminate in cylindrical conidiogenous cells with long, cicatrized sympodial rachides. Sympodial conidia are clavate, about 3×7 µm. Hyphae show early disarticulation into rectangular arthroconidia.

Formation of ascospores: Gametangia are formed at opposite sides of hyphal septa. Asci are subglobose to broadly cylindrical, (10–12)×(11–20) µm, and mostly contain 4 ascospores. Ascospores are subhyaline, ellipsoidal to subcylindrical, 4.5×(6–7) µm, and with a slime sheath 1.2–1.6 µm thick (Fig. 74). The species is homothallic.

Fermentation: absent.

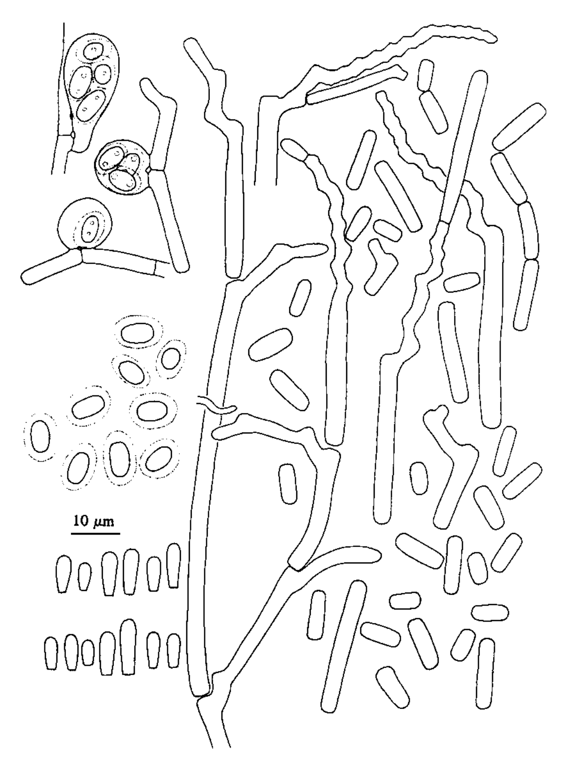


Fig. 74. *D. spicifer.* Asci with liberated ascospores, CBS 244.85; hyphae with sympodial rachides, clavate sympodial conidia and cylindrical arthroconidia, CBS 244.85, MEYA, 22°C.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	+
Arbutin	+		

Co-Q: Not determined.

Mol% G+C: 30.6, CBS 244.85 (T_m: Guého et al. 1987a).

Origin of the strain studied: CBS 244.85 from cactus rot, Arizona, Lachance.

Type strain: CBS 244.85.

Comments: The species is close to *D. capitatus*, but differs by its ability to grow with cellobiose, salicin and arbutin. Both species form sympodial rachides. Ascospores are rather abundant in the homothallic strain CBS 244.85, but scarce in the heterothallic species, *D. capitatus*.

The recent finding of strains from human blood, physiologically identical to *D. spicifer* but without ascospores, complicates the distinction of taxa within the *D. capitatus* group. Guého et al. (1987a) noted that the distinction of three taxa was justified on the basis of DNA/DNA reassociation experiments. The blood cultures may belong to either *D. capitatus* or *Geotrichum clavatum.* The latter species grows with salicin, arbutin and cellobiose, but the diagnosis should be expanded to accommodate strains with sympodial rachides. The strain described by Mahul et al. (1989) from a systemic infection in a patient with leukemia also fits *G. clavatum.* The type of *D. spicifer,* an insect-associated strain on *Opuntia* rots, occupies an ecological niche quite different from that of *D. capitatus.*

29.13. *Dipodascus tetrasperma* (Macy & M.W. Miller) von Arx (1977a)

Synonyms:

Endomyces tetrasperma Macy & M.W. Miller (1971)

Zendera tetrasperma (Macy & M.W. Miller) Redhead & Malloch (1977)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 22 mm in diameter, whitish, smooth and with finely feathered margins. Expanding hyphae are stiff, 7–9 μm wide, branched at acute angles, and, as they become older, disarticulate into short cylindrical arthroconidia (7–9)×(12–20) μm.

Formation of ascospores: Gametangia are formed with groups on opposite sides of septa. Asci are subglobose, 8–12 μm in diameter, and contain 2–4 ascospores. Ascospores are hyaline, broadly ellipsoidal, (4–5)×(6–7) μm and with a slime sheath 1.5 μm thick (Fig. 75). The species is homothallic.

Fermentation:

Glucose	+	Maltose	–
Galactose	–	Lactose	–
Sucrose	–	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

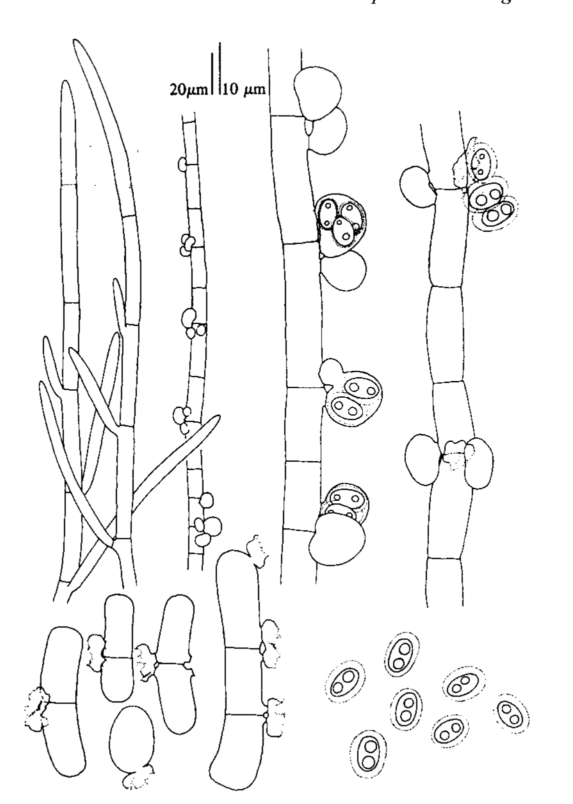


Fig. 75. *D. tetrasperma,* CBS 765.70, MEYA, 22°C. Expanding hyphae with gametangia, asci liberating ascospores and disarticulated hyphae with remains of asci.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	+

Co-Q: Not determined.

Mol% G+C: 38.8, CBS 765.70 (BD: Guého et al. 1985); 38, CBS 765.70 (T_m: Guého 1979).

Origin of the strain studied: CBS 765.70 (ATCC 22540), from wet conveyor at prune dehydration plant, Miller, California.

Type strain: CBS 765.70.

Comments: The species is close to *D. magnusii* but distinguished from it by the asci which form laterally alongside main branches, whereas those of *D. magnusii* are formed terminally on lateral branches.

Comments on the genus

Kurtzman and Robnett (1995) compared described species of *Dipodascus* from the extent of nucleotide divergence in the 5′ end of large subunit rDNAs. Phylogenetic analysis of these data divided *Dipodascus* into two subclades, one of which included species of *Galactomyces* (see Fig. 170, p. 385).

30. *Endomyces* Reess

G.S. de Hoog

Diagnosis of the genus

Colonies on natural substrates are white, floccose, pseudomycelial or with true hyphae. Conidia, if present, are sympodially produced in short chains. Species are homothallic. Asci are subglobose to clavate, thin-walled and open by a rupture at the apex. Ascospores are 2–12 per ascus, hyaline, ellipsoidal or unilaterally flattened and helmet-shaped in outline due to a unilateral slimy brim. Species are parasitic on mushrooms.

Type species

Endomyces decipiens Reess

Species accepted

1. *Endomyces cortinarii* Redhead & Malloch (1977)
2. *Endomyces decipiens* Reess (1870)
3. *Endomyces polyporicola* (T. Schumacher & Ryvarden) de Hoog, M.Th. Smith & Guého (1986)
4. *Endomyces scopularum* Helfer (1991)

Key to species

1.	a Asci upright, produced on creeping hyphae; parasitizing Aphyllophorales	*E. polyporicola*:	p. 195
	b Asci formed in whorls laterally on hyphae; parasitizing Agaricales → 2		
2(1).	a Asci containing 2 ascospores → 3		
	b Asci mostly containing 4 ascospores; on *Armillaria*	*E. decipiens*:	p. 194
3(2).	a Species parasitizing *Cortinarius*	*E. cortinarii*:	p. 194
	b Species parasitizing *Tricholoma*	*E. scopularum*:	p. 195

Systematic discussion of the species

30.1. *Endomyces cortinarii* Redhead & Malloch (1977)

Colonies on natural substrates: Colonies are white and floccose. Hyphae are hyaline, about 1 μm wide, profusely branched, and slightly constricted at the septa. Conidia are sympodially produced in short chains, and are hyaline and clavate, (1.0–2.0)×(5–15) μm. Asci have crozier-like appendages at the base, are solitary or in small groups alongside hyphae, thin-walled, subglobose, (5.5–6.5)×(7.5–12.0) μm, and contain two ascospores. Ascospores are ellipsoidal, unilaterally flattened, (3.5–4.0)×(4.0–5.5) μm, and with a prominent slimy brim at the flattened side (Fig. 76).

Origin of the strain studied: TRTC 47587, dried specimen on old carpophore of *Cortinarius huronensis*, Ontario, Canada.

Type specimen: TRTC 47587, on an agaric.

Comments: The species is only known from a single collection; it has not been cultured. The connection with the sympodial anamorph has not unambiguously been proven. The asci, having crozier-like appendages and containing two ascospores, are characteristic for the species.

30.2. *Endomyces decipiens* Reess (1870)

Colonies on natural substrates: Colonies are white and floccose. Hyphae are hyaline, 2–3 μm wide, cylindroidal or with inflated cells and constricted at the septa. Hyphae terminally disarticulate into separate cells. Asci are in small clusters just below septa and are subglobose to

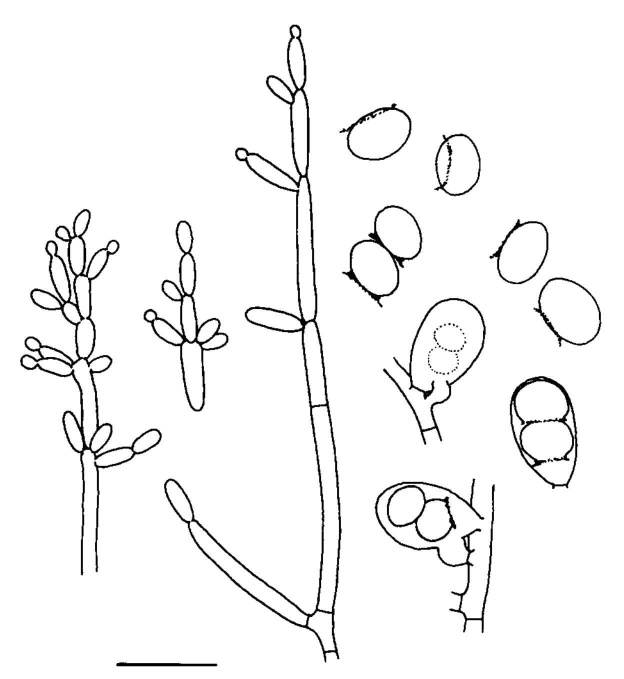


Fig. 76. *E. cortinarii*, TRTC 47587. Asci and ascospores on the natural substrate. Bar = 10 μm.

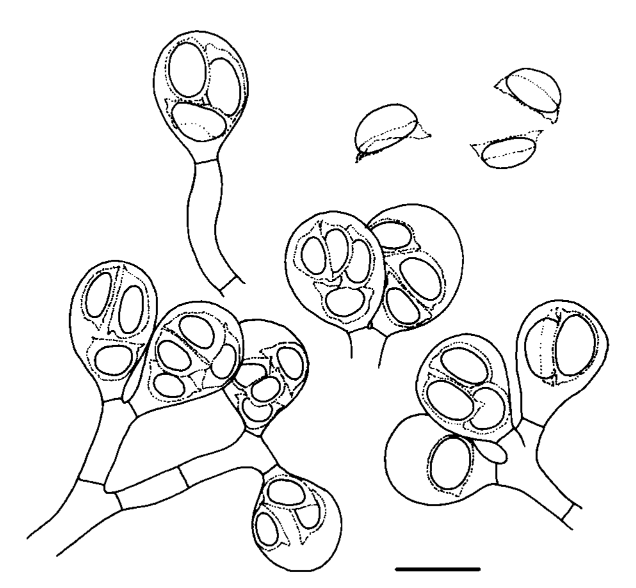


Fig. 77. *E. decipiens*, neotype TRTC. Asci and ascospores on the natural substrate. Bar = 10 µm.

clavate, (10–14)×(14–19) µm, thin-walled and contain 4 ascospores. Ascospores are broadly ellipsoidal, unilaterally flattened, (5.0–6.5)×(7.0–8.5) µm, and with a thin slime sheath which forms a brim at the flattened spore side (Fig. 77).

Origin of the specimen studied: Rehm Ascom. 1050 (TRTC), on *Armillaria mellea*, Krieger, Germany.

Neotype specimen: Rehm 1050, on an agaric.

Comments: The complicated nomenclatural history of this species was discussed by Redhead and Malloch (1977) who indicated a neotype specimen maintained at the TRTC herbarium in Toronto, Canada.

Endomyces decipiens has not been grown in culture. Similar to *Dipodascus armillariae* W. Gams, with which it has often been confused (Gams 1983), the species can be found as a parasite on carpophores of *Armillaria* (Agaricales) in autumn. A strain, mentioned by Hausner et al. (1992) as *E. decipiens* was reidentified by Kurtzman & Robnett (1995) as *Dipodascus armillariae*.

30.3. *Endomyces polyporicola* (T. Schumacher & Ryvarden) de Hoog, M.Th. Smith & Guého (1986)

Synonym:

Dipodascus polyporicola T. Schumacher & Ryvarden (1981)

Colonies on natural substrates: Colonies are whitish and hamper development of the host carpophore. Asci are single or in clusters, clavate to cylindroidal, (6–8)×(15–28) µm, and contain 4–6 ascospores which are liberated by apical deterioration of the ascus. Ascospores are hyaline, ellipsoidal, unilaterally flattened, (3–5)×(4.5–6.0) µm, and with a thin slime sheath which forms a brim at the flattened spore side (Fig. 78).

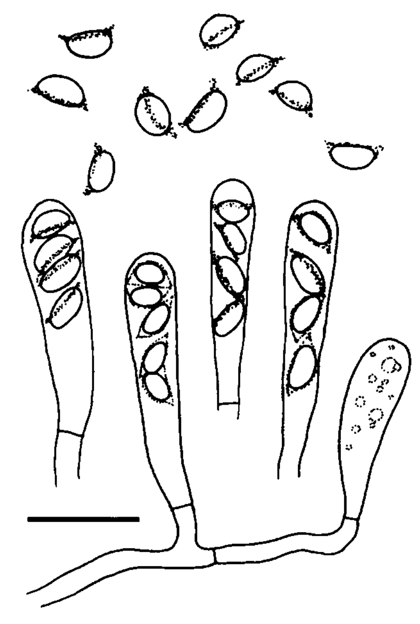


Fig. 78. *E. polyporicola*, CBS-H 1225. Asci and ascospores on the natural substrate. Bar = 10 µm.

Origin of the strain studied: CBS-H 1225, on polypore *(Piptoporus soloniensis)*, Schumacher, Thailand.

Isotype specimen CBS-H 1225, on a polypore.

Comments: The species was reported as a parasite on developing carpophores of *Piptoporus soloniensis* (Aphyllophorales). Due to the abundant overgrowth by the parasite, basidia were totally lacking from the host hymenium and only asci were found (Schumacher and Ryvarden 1981).

De Hoog et al. (1986) found conidia produced sympodially in low abundance, which possibly represented the anamorph of *E. polyporicola*. This sympodial rather than arthric anamorph, and the unilaterally hat-shaped ascospores favor the classification of this species in *Endomyces* rather than in *Dipodascus*. The species has as yet not been cultured.

30.4. *Endomyces scopularum* Helfer (1991)

Colonies on natural substrates: Colonies are white and floccose. Hyphae are hyaline, 1–2.5 µm wide, profusely branched, and slightly constricted at the septa. Conidia are sympodially produced in short chains, and are hyaline, clavate and about 1.5×5 µm. Asci have crozier-like appendages at the base, are solitary or in small groups alongside hyphae, thin-walled, subglobose, (5.5–8)×(7–9) µm, and contain two ascospores. Ascospores are ellipsoidal, unilaterally flattened, (4–5.5)×(6.5–9) µm, and separated by a unilateral brim (Fig. 79).

Type specimen: PBR M 13/84, on an agaric.

Comments: No material was available for study. Judging from the description given by Helfer (1991), the species is very close to *E. cortinarii*, also having a fragile, *Candida*-like anamorph with clavate conidia. The ascospores in *E. scopularum* were described

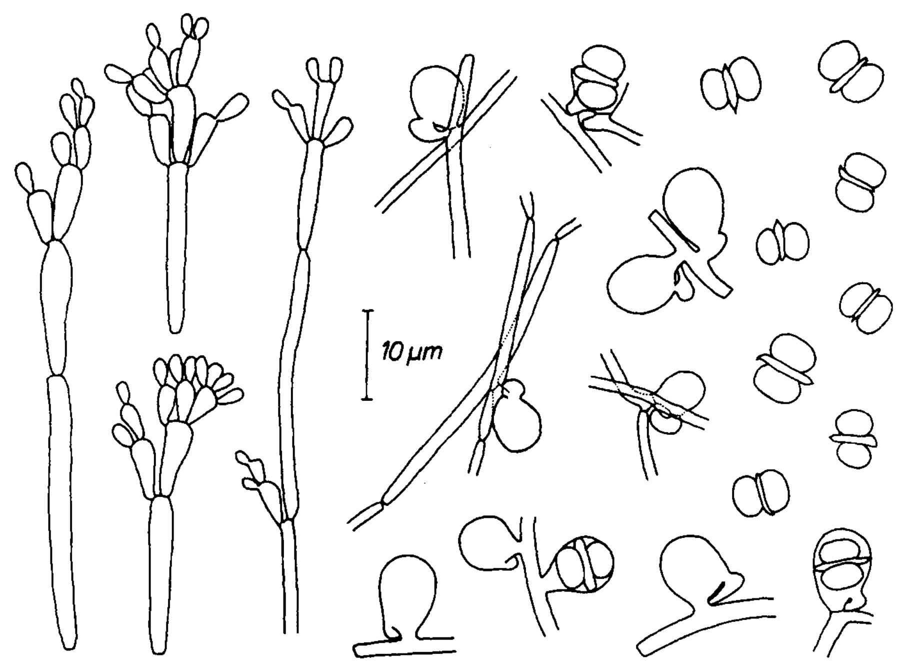


Fig. 79. *E. scopularum,* anamorph, asci and ascospores on the natural substrate. From Helfer (1991).

as lining each other with their brims, while this is less strictly the case in *E. cortinarii*. Three strains labeled *Endomyces scopularum* deposited in the CBS culture collection show an expanding, *Sporothrix*-like anamorph with conidial denticles. For one of these, CBS 131.86, partial rDNA sequences were determined by Kurtzman and Robnett (1995) who found it to cluster in the Euascomycetes. Possibly *Calcarisporium*-like contaminants were isolated rather than *Endomyces* strains.

Comments on the genus

A further species, *Endomyces parasiticus* Fayod (1885), has been described as a parasite on carpophores of *Tricholomopsis rutilans* and has not been reported since. The type specimen in the Herbarium of Geneva contains the original drawing but no specimen. The species formed small synnemata on the gills of the agaric and had monopodial, broadly clavate asci. The ascospore shape was described as ellipsoidal, without brim.

31. Endomycete-like genera of mycoparasitic fungi

Helicogonium W.L. White, *Myriogonium* Cain,
Trichomonascus H.S. Jackson, *Phialoascus* Redhead & Malloch

D. Malloch and G.S. de Hoog

Contents

31.1. *Helicogonium* W.L. White

Diagnosis of the genus

The genus is mycoparasitic. Colonies on natural substrates are composed of branched hyphae. Antheridia are undifferentiated; the ascogonium is loosely coiled and inflates unilaterally to produce a single ascus. Asci are clavate and contain 8 ascospores which are continuous with the ascogonium and open by distal deterioration. Ascospores are hyaline, thin-walled, fusiform, one- or two-celled, and bud within the ascus.

Type species

Helicogonium jacksonii W.L. White

Species accepted

1. *Helicogonium jacksonii* W.L. White (1942)

Systematic discussion of the species

31.1.1. *Helicogonium jacksonii* W.L. White (1942)

Colonies on natural substrates: Colonies have a scanty mycelium composed of branched hyphae (1.8–2.5) µm in diameter. The antheridium is an undifferentiated hyphal cell; the ascogonium is loosely coiled and inflates unilaterally to produce a single ascus. Asci contain 8 ascospores and are sessile, clavate, (7.5–10)×(28–45) µm, slightly constricted at the base but continuous with the ascogonium. The wall is slightly thickened at the non-amyloid apex and opens by apical deterioration. Ascospores are hyaline, thin-walled, fusiform, (2.5–3.0)×(7.5–11) µm, one- or two-celled, and bud within the ascus. Secondary budding cells are elongate, (1.5–2.0)×(3.0–4.5) µm, and finally fill the ascus (Fig. 80).

Origin of the specimens studied: TRTC 12919, corticiaceous fungus on *Ceraceomyces sublaevis* on coniferous wood, Ontario, Canada, Skolko; on the same substrate from Ontario, Jackson/White; from Russia, Parmasto.

Type specimen: TRTC 12919.

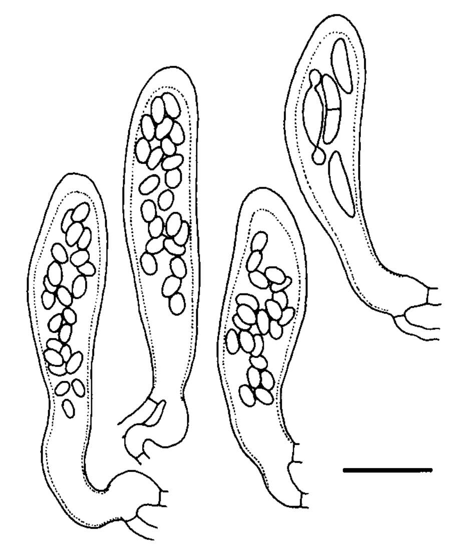


Fig. 80. *Helicogonium jacksonii*, TRTC 12919, on natural substrate. Asci with fusiform ascospores and budding within the ascus. Bar = 10 µm.

Comments: The species is only known from herbarium collections from the Northern Hemisphere. It was invariably found inside the hymenium of *Athelia sublaevis*, formerly known as *Corticium microsporum*. The host is not significantly affected by the parasite. Asci are dispersed among normally sporulating basidia. Each ascus formation is preceded by an anisogamous fusion of hyphal cells, the fusion product immediately growing out with an ascus. Croziers are absent and hence only single asci are formed after each sexual event.

Except for the budding cells within the ascus no anamorph is produced.

31.2. *Myriogonium* Cain

Diagnosis of the genus

The genus is mycoparasitic. Colonies on natural substrates are composed of branched hyphae. Gametangia are undifferentiated; asci are formed after fusion of terminal hyphal cells; subsequent asci are produced in a cymose arrangement. Asci are clavate and contain 8 ascospores. They are separated from the crozier by a septum, which opens by distal deterioration. Ascospores are hyaline, thin-walled, elongate, and one-celled.

Type species

Myriogonium odontiae Cain

Species accepted

1. *Myriogonium odontiae* Cain (1948)

Systematic discussion of the species

31.2.1. *Myriogonium odontiae* Cain (1948)

Colonies on natural substrates: Colonies are immersed in the host hymenium and composed of hyaline, amyloid, branched, strongly contorted hyphae which measure (1.0–2.5) μm. Terminal cells become somewhat swollen and fuse; one of the cells grows out into a crozier and the penultimate cell develops into an ascus. Subsequent crozier formation leads to cymosely arranged groups of asci. Asci are clavate, (5–7)×(18–25) μm, and contain 8 ascospores which are separated from the crozier by a septum. The wall is thickened at the non-amyloid apex and opens by a distal deterioration. Ascospores are hyaline, thin-walled, clavate to nearly cylindroidal, (1.2–1.7)×(4.5–6.0) μm, and are one-celled (Fig. 81).

Origin of the specimen studied: TRTC 51336, on the corticiaceous fungus *Dacryobolus sudans* on rotten conifer wood, Austria, Litschauer.

Type specimen: TRTC 51336.

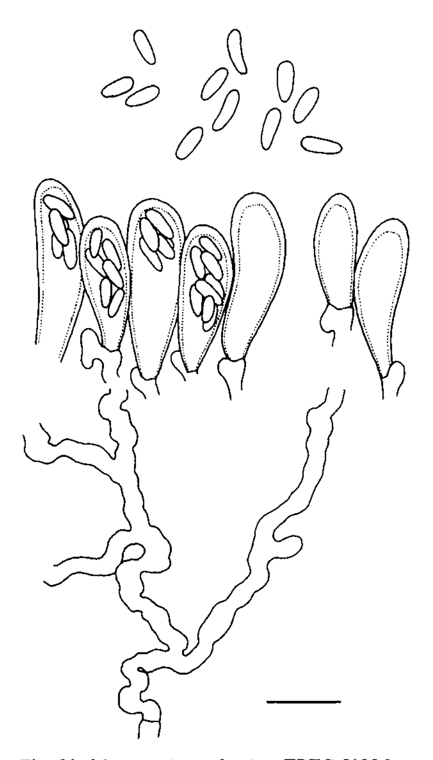


Fig. 81. *Myriogonium odontiae*, TRTC 51336, on natural substrate. Asci and ascospores; amyloid, contorted hyphae in host tissue. Bar = 10 μm.

Comments: The fungus is only known from a single herbarium specimen. The parasite grows with dispersed hyphae into the hymenium of the host without causing significant damage. *M. odontiae* invades large areas of the host fungus, occurring between normally sporulating basidia, but remaining absent from the sterile host spines which extend from the hymenium. With Melzer's reagent, the hyphae, but not the asci, show a significant amyloid reaction. In older areas the parasite becomes more abundant and then the basidiomycete tissue may be entirely replaced by the parasite. The parasite's hyphae are strongly contorted and hence septa are observed with difficulty. From initial fusion cells, the fungus spreads out with a series of asci in a cymose arrangement; each ascus is separated from the crozier by a septum. The presence of croziers suggests a relationship to Discomycetes rather than to Saccharomycetes; the species might be compared to the mycoparasitic species *Micropyxis tribidariae* (Carter and Malloch 1982). No anamorph is produced.

31.3. *Trichomonascus* H.S. Jackson

Diagnosis of the genus

The genus is mycoparasitic. Asci are immersed in host tissue and are formed terminally on undifferentiated hyphae. The initial cell cuts off a dome-shaped distal cell which is subsequently connected with the supporting hypha and develops into a sterile seta that reaches the surface of the host hymenium. The penultimate cell develops into an ascus containing one-celled ascospores.

Type species

Trichomonascus mycophagus H.S. Jackson

Species accepted

1. *Trichomonascus mycophagus* H.S. Jackson (1947)

Systematic discussion of the species

31.3.1. *Trichomonascus mycophagus* H.S. Jackson (1947)

Formation of ascospores: Asci are formed terminally on undifferentiated hyphae which are deep in host tissue. They are initially hyaline, thick-walled ellipsoidal cells, 15×10 µm, with a dome-shaped distal cell that is soon cut off. The distal cell is subsequently connected with the hypha immediately below the initial ascus. The connecting element is inflated to 4 µm in its distal portion. The ultimate cell grows out with a sterile seta 2×(60–75) µm, terminally becoming corkscrew-shaped and reaching the surface of the host hymenium. The penultimate cell develops into an ascus containing four one-celled ascospores. Ascospores are subhyaline, long-ellipsoidal to fusiform, and measure 3×(7–8) µm (Fig. 82).

Origin of the specimens studied: TRTC 21820, on the corticiaceous fungus *Radulomyces confluens* on balsam fir wood *(Abies balsamea)*, Ontario, Canada, Jackson; TRTC 32120, Ontario, Canada, Cain.

Type specimen: TRTC 21820.

Comments: The species is only known from dried herbarium collections. The karyological events leading to the formation of asci and the role of the corkscrew-shaped extensions, referred to by Jackson (1947) as 'trichogynes', can therefore only be speculated upon. The distal cell seems to be fertilized by a lateral extension and thus functions as an ascogonium, which develops a single ascus after septation. The taxonomic relationship of this peculiar fungus remains unknown (Benny and Kimbrough 1980). TRTC 32120 also contains a *Calcarisporium* anamorph with the same hyphal structure as *Trichomonascus*, but the connection between the two fungi could not unambiguously be verified.

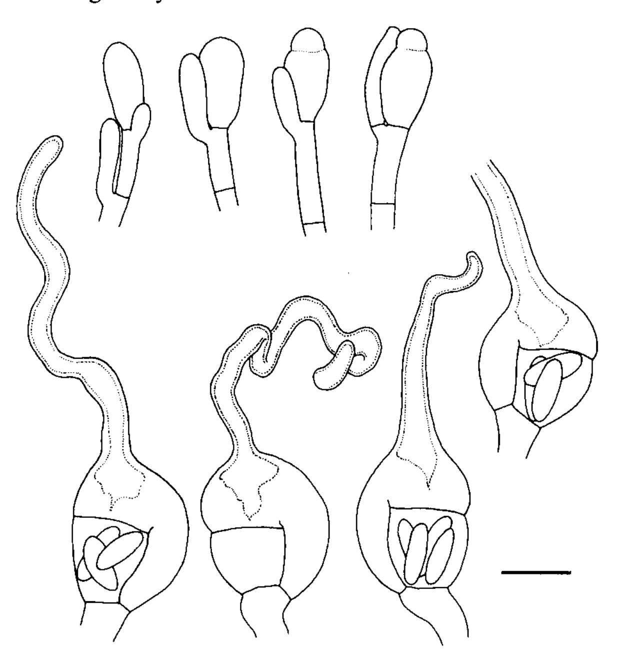


Fig. 82. *Trichomonascus mycophagus*, TRTC 21820, on natural substrate. Young ascogonium fertilized by lateral, tubular antheridium; mature asci with ascospores, crowned with corkscrew-shaped ultimate cell. Bar = 10 µm.

31.4. *Phialoascus* Redhead & Malloch

Diagnosis of the genus

The genus is mycoparasitic. Hyphae are hyaline and branched. Ascophores are erect, hyaline, moderately thick-walled and tapering towards the apex. Asci are single, terminal on ascophores, open by apical deterioration, and eventually proliferate with new asci. Ascospores are hyaline, one-celled, and enclosed in a thin gelatinous sheath.

Type species

Phialoascus borealis Redhead & Malloch

Species accepted

1. *Phialoascus borealis* Redhead & Malloch (1977)

Systematic discussion of the species

31.4.1. *Phialoascus borealis* Redhead & Malloch (1977)

Formation of ascospores: Hyphae are immersed, hyaline, branched, and (1.5–2.0) µm wide. Ascophores are one-celled, scattered, and project from and are perpendicular to the host's hymenium. They are hyaline, moderately thick-walled, taper towards the apex, frequently bear a clamp-like structure at the base, and measure (45–77) µm long, (3.0–3.5) µm wide above, (5–7) µm wide at the base. Asci are single, terminal on ascophores, hyaline, thin-walled, ellipsoidal to clavate, (6.0–6.5)×(14–18) µm, and open by apical deterioration. They contain four ascospores. Often new asci are formed by percurrent proliferation. Ascospores are hyaline, ellipsoidal and unilaterally flattened to bean-shaped, (2–4)×(3.5–7.5) µm, one-celled, and enclosed in a thin gelatinous sheath (Fig. 83).

Origin of the specimen studied: TRTC 47588, on a carpophore of *Cortinarius huronensis*, Ontario, Canada, Redhead and Malloch.

Type specimen: TRTC 47588.

Comments: Diploidization of ascophores takes place at the base of the ascophores in a fashion similar to fertilization in *Helicogonium* and *Dipodascus*. Each sexual event results in a single ascophore. As in *Cephaloascus,* each ascophore may produce several asci. The asci of *Phialoascus* proliferate percurrently and hence resemble those of *Ascoidea.*

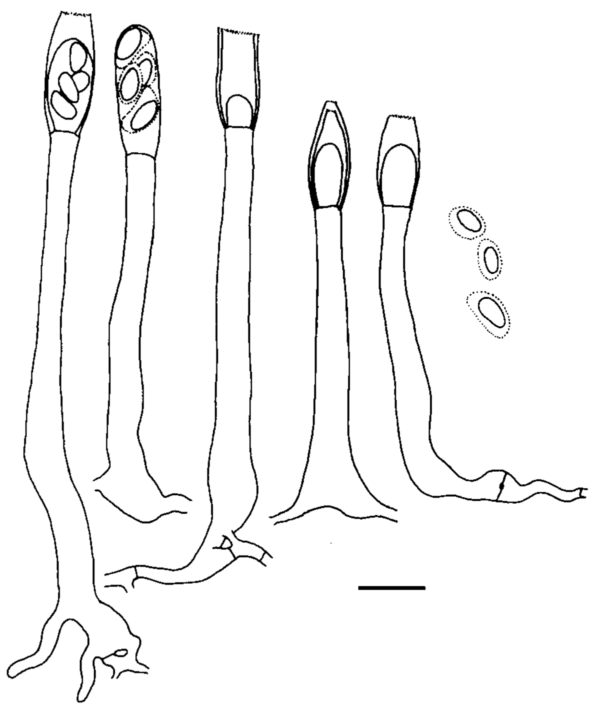


Fig. 83. *Phialoascus borealis*, TRTC 47588, on natural substrate. Ascophores with terminal, percurrent asci-liberated ascospores. Redrawn from Redhead and Malloch (1977). Bar = 10 µm.

The spores accumulate at the open ends of the asci as sticky balls, suggesting insect dispersal (Redhead and Malloch 1977). No anamorph is known.

32. *Eremothecium* Borzi emend. Kurtzman

G.S. de Hoog, C.P. Kurtzman, H.J. Phaff and M.W. Miller

Diagnosis of the genus

Budding cells are absent or present, and when present, budding is multilateral on a narrow base. Cells are globose, ovoidal, ellipsoidal or cylindroidal. Enteroarthric conidia are infrequently produced by one species. Pseudohyphae and true hyphae are generally present. Colonies are smooth or floccose and white, grayish or yellow in color.

Asci, which become deliquescent, form 8–32 ascospores that are fusiform or acicular. Ascospores may have a central septum, and those of some species have a tapered, terminal extension of the cell wall.

Sugars are fermented by some species. Nitrate is not assimilated. Coenzyme Q may have 5, 6, 7, 8, or 9 isoprene units in the side chain, some of which are in minor proportions. Diazonium blue B reaction is negative.

Type species

Eremothecium cymbalariae Borzi

Species accepted

1. *Eremothecium ashbyi* (Guilliermond ex Routien) Batra (1973)
2. *Eremothecium coryli* (Peglion) Kurtzman (1995)
3. *Eremothecium cymbalariae* Borzi (1888)
4. *Eremothecium gossypii* (Ashby & Nowell) Kurtzman (1995)
5. *Eremothecium sinecaudum* (Holley) Kurtzman (1995)

Key to species

1.	a Ascospores are curved and sickle-like in appearance	*E. ashbyi*:	p. 201
	b Ascospores are linear in appearance → 2		
2(1).	a Ascospores have a long, whip-like terminal appendage	*E. coryli*:	p. 202
	b Ascospores do not have whip-like appendages → 3		
3(2).	a Ascospores are narrowly triangular in side view with one needle-like end	*E. cymbalariae*:	p. 204
	b Ascospores are needle-shaped; length is greater than 20 µm	*E. gossypii*:	p. 204
	c Ascospores are needle-shaped; length is ca. 10 µm	*E. sinecaudum*:	p. 205

Systematic discussion of the species

32.1. *Eremothecium ashbyi* (Guilliermond ex Routien) Batra (1973)

Synonyms:
Eremothecium ashbyi Guilliermond (1935) nom. inval.
Crebrothecium ashbyi Guilliermond ex Routien (1949)
?*Spermophthora gossypii* Ashby & Nowell (1926)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 5 mm in diameter, initially tan, but later locally excreting riboflavin into the medium. Colonies are dry, initially smooth, but later develop deep radial furrows and have sharp, lobed margins. Budding cells are absent. Hyphae are dichotomously branched, 2–6 µm wide, sparingly septate, and the cells contain yellow, needle-shaped crystals. Enteroarthric conidia are rarely produced.

Growth on the surface of assimilation media: Thin pellicles are formed by some strains.

Formation of ascospores: Asci are ellipsoidal to fusiform (10–18)×(50–110) µm, and mostly intercalary and in long chains. Each ascus forms 8–16 ascospores which are liberated at maturity. The ascospores are evenly distributed in the ascus, and are hyaline, narrow, sickle-shaped, rounded at the tip, but with a basal spine (Fig. 84).

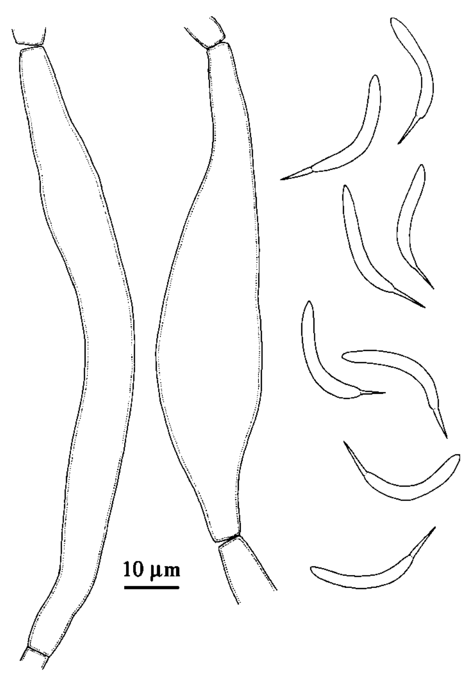


Fig. 84. *E. ashbyi*, CBS 269.75. Intercalary, disarticulating asci and sickle-shaped ascospores with cellular basal spines, after 1 week, YM agar, 25°C.

The spore body measures (2.0–3.5)×(20–30) μm, and the spine is 2–10 μm long. The species appears to be homothallic.

Ascosporulation was observed on YM agar after 1 week at 25°C.

Fermentation:

Glucose	v	Lactose	–
Galactose	–	Raffinose	v
Sucrose	v	Trehalose	v
Maltose	v		

Assimilation (in yeast nitrogen base + 0.1% yeast extract):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+/l	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	w/–
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 6, CBS 106.43 and 5 additional strains (Yamada et al. 1987b).
Mol% G + C: Not determined.

Origin of the strains studied: CBS 106.43 (ex CBS 204.36 = NRRL Y-1360), from cotton, Sudan, Massey and Guilliermond; from cotton (1); from plant-parasitic insect (1).

Authentic strain: CBS 106.43.

Comments: The name *Eremothecium ashbyi* was introduced by Guilliermond (1935) without a Latin diagnosis, but the taxon was validated by Routien (1949) in the description of *Crebrothecium*. The species has been well illustrated and described by Guilliermond (1936), Mukerji (1968) and Batra (1973). The species causes cotton boll rot in various species of *Gossypium* and induces cankers on citrus fruits. *E. ashbyi* and *E. gossypii* are often used for the industrial production of riboflavin.

32.2. *Eremothecium coryli* (Peglion) Kurtzman (1995)

Synonyms:
Nematospora coryli Peglion (1897)
?*Nematospora lycopersici* Schneider (1917)
?*Nematospora phaseoli* Wingard (1922)
Nematospora nagpuri Dastur (Dastur & Singh 1930)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoid, elongate, globose and generally polymorphic. They occur singly, in pairs, or in small chains. The relative proportions of cells of different shapes vary from strain to strain. The size of the ovoid and globose cells varies between (1.7–13.9)×(4.2–14.0) μm. A butyrous sediment is present; some granular growth may occur.

Growth on 10% malt agar: After one month at 25°C, the streak culture is light cream colored, usually abundantly wrinkled but sometimes coarsely folded, glossy, pasty or membranous, slightly raised, and the border is fringed with mycelium.

Dalmau plate culture on corn meal agar: True hyphae with few septa are present. Arthroconidia are not formed. Pseudomycelium is usually abundant (Fig. 85). The blastoconidia are ovoid or cylindrical and form short chains in verticillated positions.

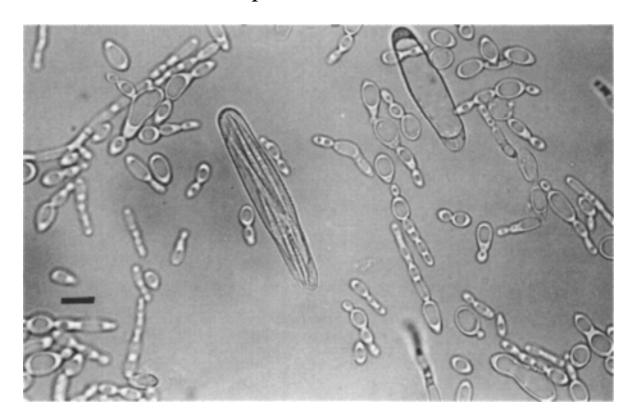


Fig. 85. *E. coryli*, CBS 2608. Ascus on V8 agar after 2 days at 20°C. Bar = 10 μm.

Formation of ascospores: Conjugation preceding ascospore formation is not apparent. Asci are long, curved cylindrical cells with rounded ends, or they are sometimes of irregular shape. Usually eight spores are formed per ascus (Fig. 85). Asci with two to four spores occasionally occur; rarely, 12 or 16 spores are present. Asci release the spores very soon after maturity. The spores are spindle-shaped with a long, thin, whip-like appendage at one end. SEM photomicrographs show that the surface of the spore-half carrying the appendage is smooth whereas that of the blunted half is ornamented with concentric ridges. This ornamentation is reversed from that in *E. sinecaudum.* Examination under the light microscope reveals the presence of vacuoles and a difference of refringence between the two halves of the spores. Staining with the periodic-Schiff technique shows that the wall of the half to which the whip is attached takes a deep pink shade whereas the other half remains almost unstained. When stained with the Ziehl–Neelsen or the Schaeffer–Fulton malachite green technique, the half supporting the whip-like appendage is not acid fast and does not retain the malachite green; the other half is acid fast

and retains the malachite green (do Carmo-Sousa 1970a). Electron micrographs of longitudinal sections have shown that the spores lack a septum (Kreger-van Rij 1969a). The spores are usually grouped in two bundles of four within the ascus. The whip-free ends of the spore bundles are directed towards the poles of the ascus. Germination of the spores usually takes place near the equator of the spindle on the side of the whip-supporting half and gives rise either to buds or hyphae (Fig. 86).

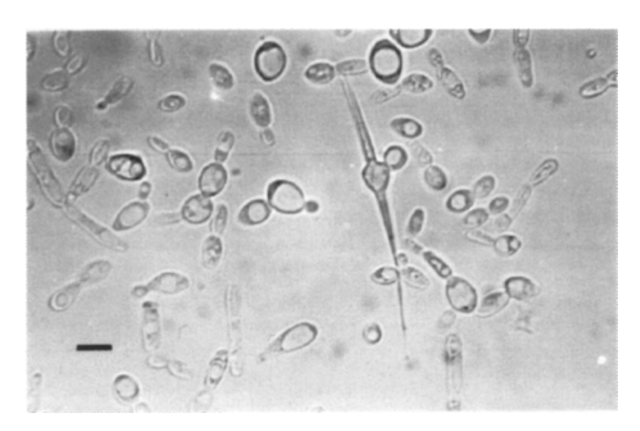


Fig. 86. *E. coryli*, CBS 2608. Vegetative cells and germinating ascospore with a bud, after 3 days on 5% malt extract agar at 25°C. Bar = 10 µm.

Ascosporulation was observed after 2–3 days on V8 agar at 20°C in five out of nine strains. Some strains also sporulated on malt agar and glucose–yeast extract–peptone agar.

Fermentation (30°C):

Glucose	+	Lactose	–
Galactose	–	Raffinose	v
Sucrose	+	Trehalose	s
Maltose	+	Soluble starch	w/–

Assimilation (30°C, in yeast nitrogen base + 0.1% yeast extract):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	–
5-Keto-D-gluconate	–	Lipase	+
Cadaverine	–	Gelatin hydrolysis	v
L-Lysine	–	Casein hydrolysis	v
Ethylamine	–	Cycloheximide 100 µg/ml	+
50% Glucose	–	Acid production	v
10% NaCl/5% glucose	–	Growth at 40°C	+
Starch synthesis	–	Growth at 45°C	–

Co-Q: 5, CBS 2608, CBS 79 (Yamada et al. 1981); 6, CBS 2599, 2600, 2601, 2604; IGC 3027 = UCD-FST 66-37; NRRL Y-1808 = UCD-FST 68-11; IFO 0658 (Y. Yamada, personal communication).

Mol% G+C: 43.2, 43.4, IGC 3027 and CBS 2608, resp. (BD: H.J. Phaff, unpublished data).

Origin of the strains studied: CBS 2608 = UCD-FST 66-36 = IFO 1220, isolated by Peglion in 1897 from diseased hazelnuts in Italy; CBS 2599 from cotton bolls, Belgian Congo; CBS 2600 from cotton bolls, U.S.A.; CBS 2601 from tomatoes, British West Indies; CBS 2604, origin not known; CBS 79, from cotton bolls, India (type strain of *Nematospora nagpuri*); IGC 3027, from *Phaseolus vulgaris*, Uganda; NRRL Y-1808, from lima beans, U.S.A.; IFO 0658, from A.J. Kluyver, origin not known.

Type strain: CBS 2608 (IFO 1220) from diseased hazelnuts in Italy (Peglion 1897).

Comments: Strains of *E. coryli* grow rather poorly in YNB media but supplementation with 0.1% yeast extract (Difco) resulted in rapid and good growth with assimilable carbon sources and no significant growth in control tubes without a carbon source. Because of the high maximum temperature for growth of this species, the fermentation and assimilation tests were conducted at 30°C, which further improved the final test results. *E. sinecaudum* responded similarly to the growth-promoting activity of yeast extract in YNB media.

The question of synonymy for *Nematospora phaseoli* and *N. lycopersici* with *E. coryli* results from the lack of authentic strains of these two species. Batra (1973) recognized *N. lycopersici* as a species based on one strain isolated from tomatoes and two from beans. One of these (CMI No. 77-422 from *Phaseolus vulgaris* in Uganda) was included in our study. Since its characteristics did not differ from the other strains, we concluded, in agreement with do Carmo-Sousa (1970a), that it is conspecific with *E. coryli*. Batra reported positive nitrate reduction whereas we were unable to obtain growth of any of the strains when nitrate was used as the sole source of nitrogen in the medium.

The alternation between haplophase and diplophase in *E. coryli* is unresolved. Koopmans (1977) made a careful cytological study of *E. coryli* during vegetative growth and sporulation, but was unable to determine at which stage a cell is committed to become an ascus. She assumed that in some enlarged cells mitotic nuclei fused to form a diploid nucleus, which underwent meiosis to eight nuclei in a greatly enlarged ascus.

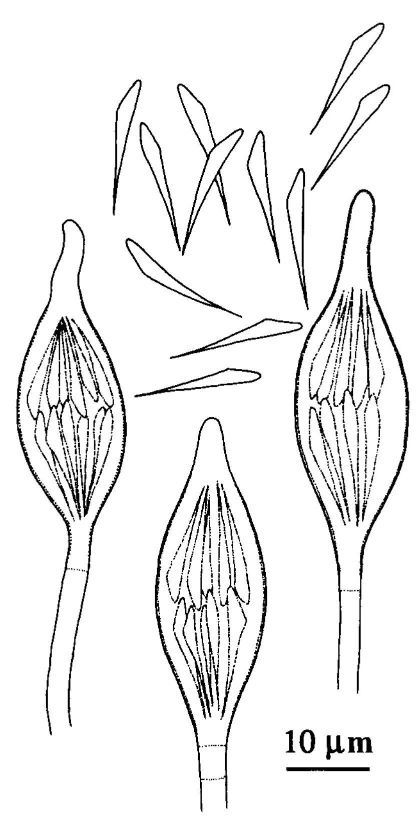


Fig. 87. *E. cymbalariae,* CBS 270.75. Terminal asci with inconspicuous basal septa; asymmetrically acicular ascospores. After 3 days on YM agar at 25°C.

E. coryli is parasitic on plants, causing a disease known as stigmatomycosis. The yeast has been found in many species of plants throughout subtropical and tropical areas of the world and is always associated with insect punctures of the plant tissue made by insects with piercing–sucking mouth parts. Frazer (1944) has made a detailed study of the infection of cotton bolls by the cotton stainer, *Dysdercus*. The spores of *E. coryli* appear to be harbored in the stylet pouches of *Dysdercus suturellus.*

32.3. *Eremothecium cymbalariae* Borzi (1888)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 20 mm in diameter, initially tan, but later may exude a yellow pigment into the medium. Colonies are dry and smooth, becoming floccose at the center, and develop a sharp, lobed margin. Budding cells are absent. Hyphae are 2–4 µm wide, fragile, and have few septa.

Growth on the surface of assimilation media: Pellicles are not formed.

Formation of ascospores: Asci are formed on hyphal tips, often without distinct basal septa, and are narrowly limoniform with a terminal elongation. They measure (12–14)×(30–65) µm, inclusive of the acuminate tip. Usually 8–16 ascospores are formed in each ascus and liberated at maturity (Fig. 87). They often adhere to one another in rhomboid aggregations. Ascospores are hyaline, narrowly triangular and with needle-shaped ends, (2)×(13–16) µm, with the broadest part just below the apex. The ascospores develop in the ascus in two opposed groups with the needle-shaped tips toward the outside. The species appears homothallic.

Ascospores were observed on YM agar after 3 days at 20–25°C.

Fermentation: absent.

Assimilation (in yeast nitrogen base + 0.1% yeast extract):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	w/–	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+/w	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	w/–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 270.75 (Yamada et al. 1987b).
Mol% G + C: Not determined.

Origin of the strain studied: CBS 270.75, from a plant-pathogenic insect, Iran, Ershad.

Type strain: CBS 270.75, designated here as the neotype.

Comments: No type specimen of *E. cymbalariae* is known to be preserved, consequently, CBS 270.75 is designated as the neotype. The species was reported as a pathogen of flax and other plants and was redescribed by Arnaud (1913) and Ashby and Nowell (1926). The near absence of septa in this fragile, purely hyphal fungus is remarkable. The physiological pattern of the strain was tested in yeast nitrogen base supplemented with 0.1% yeast extract to enhance growth reactions.

32.4. *Eremothecium gossypii* (Ashby & Nowell) Kurtzman (1995)

Synonyms:
Nematospora gossypii Ashby & Nowell (1926)
Ashbya gossypii (Ashby & Nowell) Guilliermond (1928)
Ashbia gossypii (Ashby & Nowell) Ciferri & Fragoso (Fragoso and Ciferri 1928)

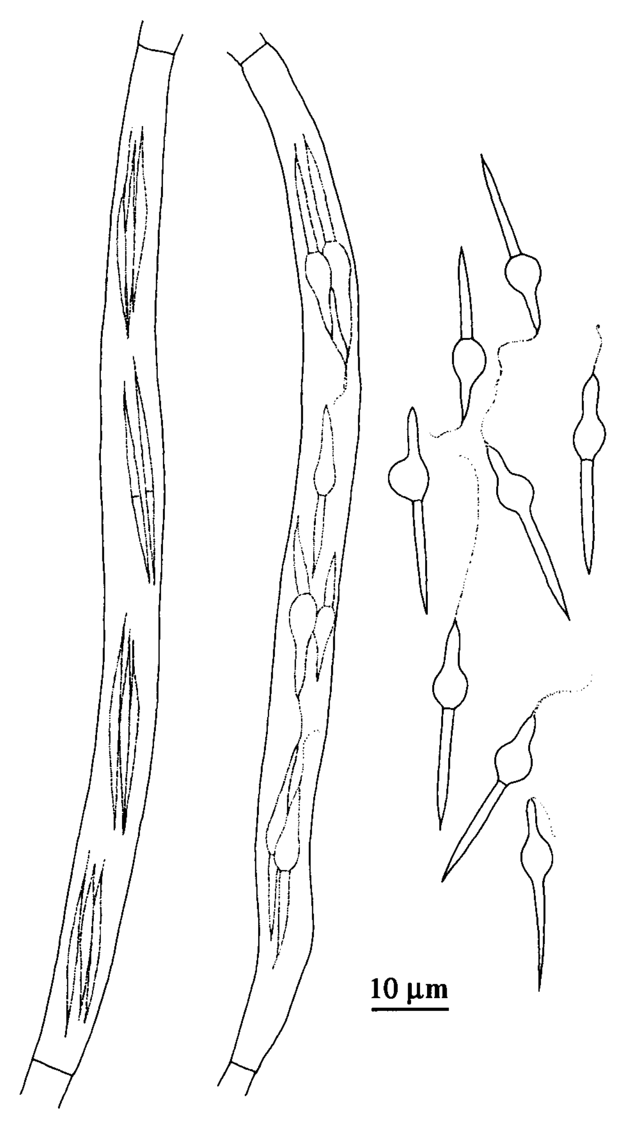


Fig. 88. *E. gossypii*, CBS 117.28. Intercalary asci in various stages of maturation, and ascospores showing swelling of the germinating cell. After 1 week on YM agar, 25°C.

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 20 mm in diameter, initially tan, but later exuding a yellow pigment into the medium. Colonies are smooth or finely floccose, moist, wrinkled at the center, and with a sharp, lobed margin. Hyphae (2–6 μm wide) branch dichotomously, and some cells contain yellow, needle-shaped crystals of riboflavin. Conidia, when present, are formed laterally on hyphae, either singly or in short chains, and are ellipsoidal, (2.5–3.0)×(4.5–7.0) μm.

Growth on the surface of assimilation media: Thin pellicles are formed by some strains.

Formation of ascospores: The asci, which are clavate to fusiform, (10–20)×(60–200) μm, are mostly intercalary and in long chains. They disarticulate at maturity and then release the ascospores. Asci contain 12–32 ascospores which are arranged in parallel packets (Fig. 88), and are hyaline, and needle- or spindle-shaped; they usually become two-celled following formation of a median septum. The ascospores, which measure (2.0–3.5)×(25–35) μm, have a terminal, filiform appendage that may be as long as 100 μm. Appendages are slimy, causing the ascospores to unite in small groups. The species appears to be homothallic.

Ascospores were observed on YM agar after 1 week at 25°C.

Fermentation: absent.

Assimilation (in yeast nitrogen base + 0.1% yeast extract):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+/w	Erythritol	–
Cellobiose	w/–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+/w
L-Arabinose	–	Citrate	w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	v
Saccharate	–	Growth at 37°C	+/w
10% NaCl/5% glucose	–		

Co-Q: 6, CBS 109.26, CBS 109.51 (Yamada et al. 1987b).
Mol% G + C: Not determined.

Origin of the strains studied: CBS 109.26, from fruit of *Asclepias* sp., Ashby, Trinidad; from cotton, South Africa (2), Trinidad (2); from unknown sources (5).

Authentic strain: CBS 109.26

Comments: Type material is not known to exist for *E. gossypii*, consequently, CBS 109.26 has been designated as an authentic strain. Pridham and Raper (1950) and Batra (1973) have provided extensive documentation of the distribution of *E. gossypii* and most other members of *Eremothecium*.

32.5. *Eremothecium sinecaudum* (Holley) Kurtzman (1995)

Synonyms:
Nematospora sinecauda Holley (Holley et al. 1984)
Holleya sinecauda (Holley) Yamada (1986b)

Growth on YM agar: After 3 days at 30°C, the cells are globose, ellipsoidal and cylindrical, and occur singly, in pairs, or in short chains. Pseudomycelium develops in older cultures. After 2 weeks the streak culture is

cream-colored, flat, semi-glossy with a lightly corrugated surface, a pasty texture, and a periphery that is entire or slightly wavy.

Growth in YM broth: After 10 days at 30°C, there is abundant sediment; a ring or pellicle is lacking. Cells are mainly cylindrical, but some are ovoidal to long ovoidal or globose or clavate, occasionally with a terminal swelling. Budding is polar (2–7)×(7–25) µm, with cells occasionally up to 30–50 µm long. Growth shows simple to extensively branched pseudomycelium.

Dalmau plate culture on corn meal agar: After 9 days at 30°C, there is very dense and extensive pseudomycelium with elongate to short cylindrical blastoconidia formed aerobically as well as under the coverslip (Fig. 89).

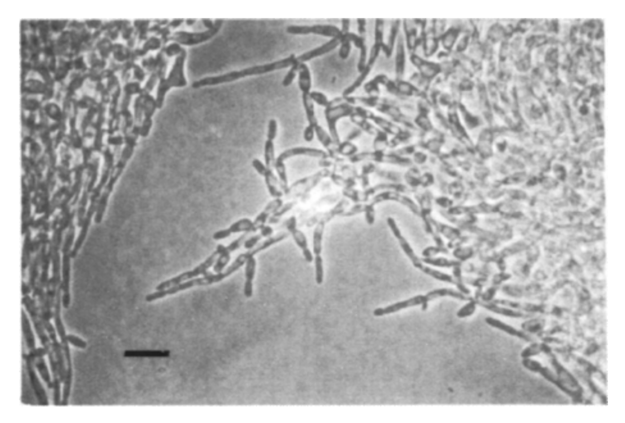


Fig. 89. *E. sinecaudum,* CBS 8199. Pseudomycelium on corn meal agar after 8 days at 30°C. Bar = 20 µm.

Formation of ascospores: Asci arise directly from enlarged irregularly shaped vegetative cells which contain two bundles or fascicles of four ascospores each. The spores are acicular or needle-shaped and lie with their blunt ends at the ascus poles, each fascicle at opposite sides of the ascus (Fig. 90). The asci are approximately 8×20 µm and the ascospores 1.3–2.5×10 µm. Spores are released from the asci upon maturity. Individual spores have a smooth surface at the blunt end, a swollen equatorial plate collar and concentric ridges on the surface of the pointed half which terminates without an appendage (see Holley et al. 1984, Figs. 89, 90). Spores germinate by swelling of the half with the blunt end.

Sporulation occurs readily on YM or malt agar at 30°C after 3 days.

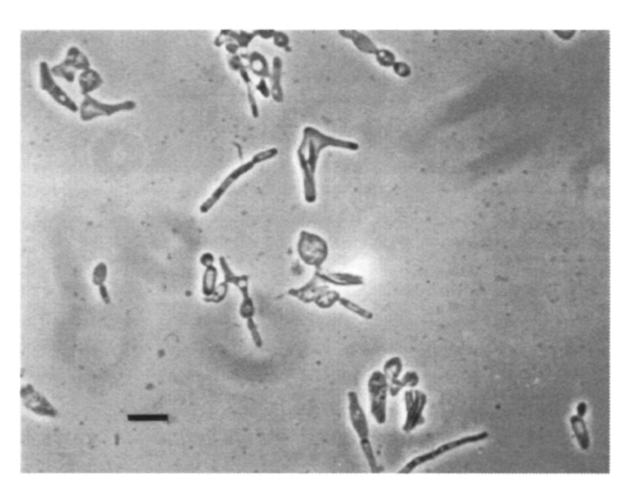


Fig. 90. *E. sinecaudum,* CBS 8199. Ascus with 2 bundles of 4 ascospores each. After 3 days on 5% malt extract agar at 30°C. Bar = 15 µm.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	w/–	Trehalose	w/–
Maltose	w/–		

Assimilation (in yeast nitrogen base + 0.1% yeast extract):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	l
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
5-Keto-D-gluconate	–	Cycloheximide 100 mg/ml	+
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–	Growth at 40°C	+
Starch formation	–	Growth at 45°C	–

Co-Q: 9 (minor, Q 8) (Yamada 1986b).

Mol% G + C: 41.8, UCD-FST 80-21 (BD: Phaff, unpublished data).

Origin of the strains studied: CBS 8199, 7233, and 7234 isolated by R.A. Holley from oriental *(Brassica juncea)* and yellow *(B. hirta)* mustard seed; UCD-FST 80-21 from mustard seed obtained from a commercial spice company, U.S.A.

Type strain: CBS 8199, isolated by Holley and Timbers (1983) from highly contaminated oriental mustard seed, *Brassica juncea,* in commercial storage.

Comments: Infection of mustard seed by a *Nematospora*-like yeast appears to be widespread (Hemmingway 1980). Holley and Timbers (1983) developed a microwave treatment to destroy contaminating yeasts inside the seed without affecting the flavor principle of mustard. Holley et al. (1984) isolated several strains of the contaminating yeast and described it as a new species of *Nematospora,*

N. sinecauda, based on the size and shape of the asci and spores and the absence of a whip-like appendage on the latter. Excellent electron micrographs of asci and spores accompanied the description. Yamada (1986b) restudied the strains that Holley had designated as *N. sinecauda.* On the basis of the presence of coenzyme Q-9 as the main ubiquinone in this species, in contrast to that of coenzyme Q-6 in most strains of *Nematospora coryli,* and the different size and morphology of their asci and ascospores, Yamada transferred *N. sinecauda* to a new genus, *Holleya,* as *H. sinecauda.* The transfer of *H. sinecauda* to *Eremothecium* is discussed under Comments on the genus.

Although Holley et al. (1984) reported the assimilation of carbon compounds in Difco yeast nitrogen base (YNB), we obtained very poor growth with this medium, especially on agar media in the presence of other yeasts. This is presumably due to the rapid drop in pH of this medium during growth and the reported sensitivity of *H. sinecauda* to pH values below 5.0. We supplemented liquid YNB with 0.1% yeast autolyzate and obtained excellent and rapid growth at 30°C with assimilable compounds, whereas controls without a carbon source gave a maximum response no greater than a weak reaction. Our results differ somewhat from those of Holley et al. (1984). Additionally, fermentation reactions differ among laboratories and this may reflect the nutrient composition of test media (Barnett et al. 1990, C.P. Kurtzman, unpublished data).

Comments on the genus

In the present treatment of *Eremothecium,* the genera *Ashbya, Holleya* and *Nematospora* have been placed into synonymy. The four genera are characterized by needle-shaped ascospores that may be linear or falcate. Ascospores of *Nematospora* differ further and are appended with a long flagellum-like terminal extension of cell wall material. The taxa are pathogenic to a variety of plant species (Batra 1973), and *Ashbya* and *Eremothecium* are used worldwide for production of riboflavin (vitamin B_2) (Kaplan and Demain 1970, Wickerham et al. 1946a).

Batra (1973) and von Arx et al. (1977) discussed the phenotypic similarities of *Ashbya, Eremothecium* and *Nematospora* and suggested that the three taxa might be congeneric. Another genus in this complex is *Spermophthora* Ashby & Nowell. There is no extant type material from *Spermophthora,* and strains of the taxon have not been isolated since its original description. Von Arx et al. (1977) suggested that *Spermophthora gossypii* Ashby & Nowell, the only known species of *Spermophthora,* represented a contaminated specimen of *Eremothecium* (*Crebrothecium* Routien) *ashbyi.* Additionally, classification of the foregoing taxa has been complicated by the perception (Lodder 1970) that genera which commonly form budding yeast cells *(Holleya, Nematospora)* are phylogenetically separate from genera that do not ordinarily form budding cells *(Ashbya, Eremothecium).*

Holleya sinecauda was first described as *Nematospora sinecauda* (Holley et al. 1984). Yamada (1986b) transferred *N. sinecauda* to the new genus *Holleya* because, unlike *Nematospora coryli,* which has ascospores with long terminal appendages and coenzyme Q with either five or six isoprene units, *N. sinecauda* forms shorter ascospores without terminal appendages and produces coenzyme Q with a mixture of eight (minor component) and nine (major component) isoprene units. Yamada and Nagahama (1991) compared partial rRNA sequences from *H. sinecauda* and *N. coryli,* and suggested that the extent of divergence was great enough to justify maintaining *Holleya* and *Nematospora* as separate genera.

Phylogenetic analysis of rRNA/rDNA nucleotide sequence divergence in selected genera of ascomycetous yeasts and yeastlike fungi has indicated that the budding yeasts and yeastlike fungi represent a group of taxa that is separate from the filamentous ascomycetes (Barns et al. 1991, Berbee and Taylor 1993, Hendriks et al. 1992, Kurtzman 1993a,c, Walker 1985a). In an extension of these studies, Kurtzman and Robnett (1994a) compared partial sequences of large and small subunit rRNAs from the type species of all cultivatable genera of ascomycetous yeasts and yeastlike fungi. Results from this study demonstrated that all budding yeasts and yeastlike fungi are members of a clade separate from the filamentous ascomycetes. The analysis further demonstrated that *Ashbya, Eremothecium, Holleya* and *Nematospora* represent closely related members of a subclade that is phylogenetically separate from the subclade that includes the genus *Metschnikowia,* which is also characterized by needle-shaped ascospores.

Taken in the context of this larger data set, Kurtzman (1995) proposed the transfer of species of *Ashbya, Holleya* and *Nematospora* to *Eremothecium,* the genus of taxonomic priority, pointing out that the five species to be included in *Eremothecium* are members of a monophyletic lineage and that the species show relatively little interspecific divergence. At odds with this interpretation is the disparity in coenzyme Q values found among the species. Some genera show no differences in coenzyme Q contents, whereas other genera, and even some species, produce two forms, but none has the wide range seen among species of the *Ashbya/Eremothecium/Holleya/Nematospora* clade. However, because rRNA/rDNA comparisons have been reliable predictors of kinship among other groups of yeasts (Kurtzman 1992a, 1994), the sequence analysis was given precedence in defining the taxonomy of this group. Additionally, Messner et al. (1995) compared other rRNA gene sequences from *N. coryli, A. gossypii* and *E. ashbyi* and concluded that the three taxa are congeneric.

Species of *Eremothecium,* as noted earlier, grow poorly in yeast nitrogen base unless it is supplemented with

0.1% yeast extract. Even with this adjustment, some variability in assimilation reactions was found among the laboratories of the authors of this chapter. Furthermore, fermentation reactions have been variable between laboratories as well as between repeats in the same laboratory. Consequently, it seems more reliable to key species of *Eremothecium* on the basis of their morphological characters.

33. *Galactomyces* Redhead & Malloch

G.S. de Hoog, M.Th. Smith and E. Guého

Diagnosis of the genus

Colonies are white, farinose or hairy, and dry; hyphae are hyaline and disarticulate into rectangular arthroconidia. Septa have micropores.

Gametangia are formed laterally on profusely septate parts of hyphae on opposite sides of septa. Asci are subspherical, containing 1 ascospore which is liberated by disintegration of the entire ascus wall. Ascospores are round, pale brownish, with a rough outer wall layer and with an exosporium and an equatorial furrow.

Fermentation is weak or absent. Nitrate is not assimilated. Extracellular starch is not produced. Diazonium blue B reaction is negative.

Anamorph genus is *Geotrichum*.

Type species

Galactomyces geotrichum (E.E. Butler & L.J. Petersen) Redhead & Malloch

Species accepted

1. *Galactomyces citri-aurantii* E.E. Butler (1988)
2. *Galactomyces geotrichum* (E.E. Butler & L.J. Petersen) Redhead & Malloch (1977)
3. *Galactomyces reessii* (van der Walt) Redhead & Malloch (1977)

Key to species

See Table 24.

Morphological key:

1. a Ascospores echinulate, longest dimensions 7–10 µm .... *G. geotrichum*: p. 210
   b Ascospores finely verrucose, longest dimensions 6–9 µm → 2

2(1). a Ascospores (5–6)×(6–7) µm; species homothallic .... *G. reessii*: p. 212
   b Ascospores (5–7)×(7–9) µm; species heterothallic .... *G. citri-aurantii*: p. 209

Physiological key:

1. a Growth without vitamins .... *G. geotrichum*: p. 210
   b Absence of growth without vitamins → 2

2(1). a Growth with ribitol or mannitol .... *G. citri-aurantii*: p. 209
   b Absence of growth with ribitol or mannitol .... *G. reessii*: p. 212

Table 24
Key characters of species in the genus *Galactomyces*

Species	Fermentation of glucose	Assimilation			Growth in vitamin-free medium	Mating system
		Ribitol	Mannitol	Glucono-δ-lactone		
Galactomyces citri-aurantii	–	+	+	v	–	Heterothallic
G. geotrichum	v	v	v	v	+	Heterothallic
G. reessii	w/–	–	–	–	–	Homothallic

Systematic discussion of the species

33.1. *Galactomyces citri-aurantii* E.E. Butler (Butler et al. 1988)

Anamorph: *Geotrichum citri-aurantii* (Ferraris) E.E. Butler

Synonyms:

Oidium citri-aurantii Ferraris (1899)

Oospora citri-aurantii (Ferraris) Saccardo & Sydow (1902)

Geotrichum citri-aurantii (Ferraris) E.E. Butler (Butler et al. 1988)

Geotrichum candidum Link var. *citri-aurantii* (Ferraris) R. Ciferri & F. Ciferri (Ciferri 1955a)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 70–80 mm in diameter, white, flat, dry, and powdery to finely hairy. Hyphae are 2.5–10.0 µm wide, with frequent dichotomous branching at the apex, with early disarticulation into cubic arthroconidia. Arthroconidia are hyaline and slightly inflating to (4–6)×(5–17) µm.

Formation of ascospores: Gametangia are formed in dense clusters laterally on fertile hyphae; gametangia are located on opposite sides of septa or on separate hyphae. Asci are subspherical, (7–12)×(6–9) µm, and contain

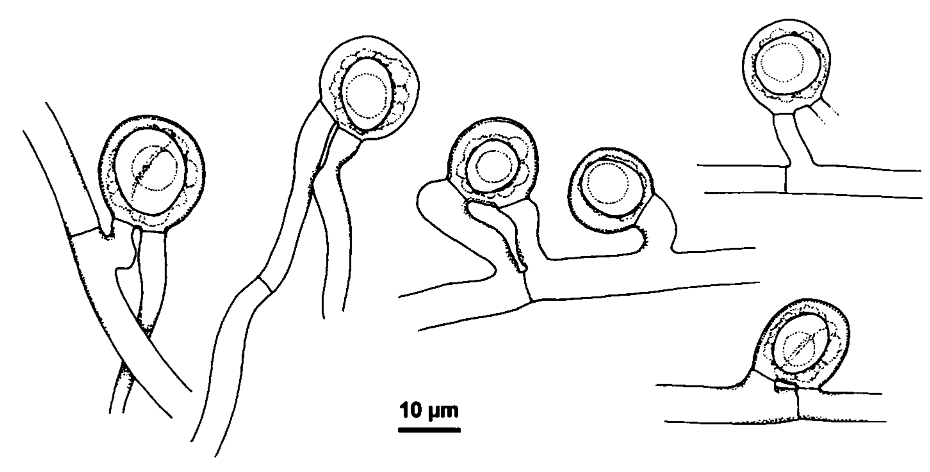


Fig. 91. *G. citri-aurantii*, CBS 175.89×176.89, PDA, 22°C, 2 weeks. Asci with mature ascospores.

one ascospore. Ascospores are broadly ellipsoidal, (5–7)×(7–9) μm, hyaline, with a smooth or verruculose inner wall and an irregular exosporium, often with a hyaline equatorial furrow and a central lipid droplet (Fig. 91). The species is heterothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Arbutin	–	Growth at 37°C	–

Co-Q: Not determined.
Mol% G+C: 37.9–39.2, 5 strains (T_m: Smith et al. 1995c).

Origin of the strains studied: CBS 175.89, from soil of an orange orchard, Zimbabwe, Butler; CBS 176.89, from soil of an orange orchard, California; from citrus (3); from soil (4).

Complementary mating types: Mating type *A1*: CBS 228.38, CBS 175.89, CBS 604.85, Butler 564B, Butler 594A; mating type *A2*: CBS 176.89, CBS 605.85, Butler 564C, Butler 594B.

Type strain: CBS 175.89×176.89, the tester strains of *Galactomyces citri-aurantii.*

Comments: The species was segregated from *Galactomyces geotrichum/Geotrichum candidum* for a taxon causing sour rot on citrus. Smith et al. (1995c) confirmed this conclusion by obtaining low DNA/DNA homology values for the species with both *Galactomyces geotrichum* and *G. reessii.*

33.2. *Galactomyces geotrichum* (E.E. Butler & L.J. Petersen) Redhead & Malloch (1977)

Anamorph: *Geotrichum candidum* Link:Fries
Synonyms:
Endomyces geotrichum E.E. Butler & L.J. Petersen (1972)
Dipodascus geotrichum (E.E. Butler & L.J. Petersen) von Arx (1977a)
Geotrichum candidum Link (1809)
Geotrichum candidum Link:Fries (1832)
Botrytis geotricha Link (1824)
Acrosporium candidum (Link) Sprengel (1827)
Torula geotricha (Link) Corda (Sturm 1829)
Mycoderma malti-juniperi Desmazières (1827)
Oidium lactis Fresenius (1850)
Oospora lactis (Fresenius) Saccardo (1886)
Oosporoidea lactis (Fresenius) Sumstine (1913)
Oidium lactis Fresenius var. *luxurians* Reiss (1854)
Oidium obtusum von Thümen (1875)
Oospora lactis (Fresenius) Saccardo var. *obtusa* (von Thümen) Saccardo (1886)
Oidium nubilum Weigmann & Wolff (1909)
Oospora nubila (Weigmann & Wolff) Berkhout (1923)
Oidium humi Mazé (1910)
Oospora humi (Mazé) Berkhout (1923)
Monilia asteroides Castellani (1914)
Oidium asteroides (Castellani) Castellani & Chalmers (1919)
Mycoderma asteroides (Castellani) Brumpt (1922)
Geotrichum asteroides (Castellani) Basgal (1931)
Oidium matalense Castellani (1915)
Mycoderma matalensis (Castellani) Brumpt (1922)
Oospora matalensis (Castellani) Berkhout (1923)
Pseudomycoderma matalensis (Castellani) Ciferri (1930b)
Geotrichum matalense (Castellani) Castellani (1932)
Pseudomonilia matalensis (Castellani) C.W. Dodge (1935)
Endomyces lactis Windisch var. *matalensis* (Castellani) Windisch (1951)
Trichosporon matalense (Castellani) Ciferri (1955b)
Oospora lactis (Fresenius) Saccardo var. *parasitica* Pritchard & Porte (1923)
Oidium suaveolens Krzemecki var. *minuta* Berkhout (1923)

Oospora fragrans Berkhout var. *minuta* Berkhout (1923)
Oospora lactis (Fresenius) Saccardo var. *exuberans* Stautz (1931)
Geotrichum matalense (Castellani) Castellani var. *chapmanii* Castellani (1932)
Geotrichum javanense Verona (1933)
Geotrichum versiforme M. Moore (1934)
Geotrichum redaellii Negroni & Fischer (1940)
Endomyces lactis Windisch (1951)
Geotrichum novakii El-Masry & Zsolt (1968)
Geotrichum pseudocandidum Saëz (1968b)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 50–80 mm in diameter, white, flat, dry, and powdery to finely hairy. Odor is insignificant or fruity. Expanding hyphae are parallel, often with dichotomous branching; the main branches are 7–12 µm wide, with firm walls, and lateral branches are 2.5–4.0 µm wide, with early disarticulation into cubic arthroconidia. Arthroconidia are hyaline and slightly inflating to (5–6) × (5–17) µm.

Formation of ascospores: Gametangia are formed laterally on densely septate parts of fertile hyphae which arise after mating; gametangia are located on opposite sides of the septa. Asci are subspherical, (7–10) × (6–9) µm, and contain one ascospore. Ascospores are broadly ellipsoidal, (6–9) × (7–10) µm, pale golden-brown, with an echinulate inner wall and an irregular exosporium, often with a hyaline equatorial furrow (Fig. 92). The species is heterothallic.

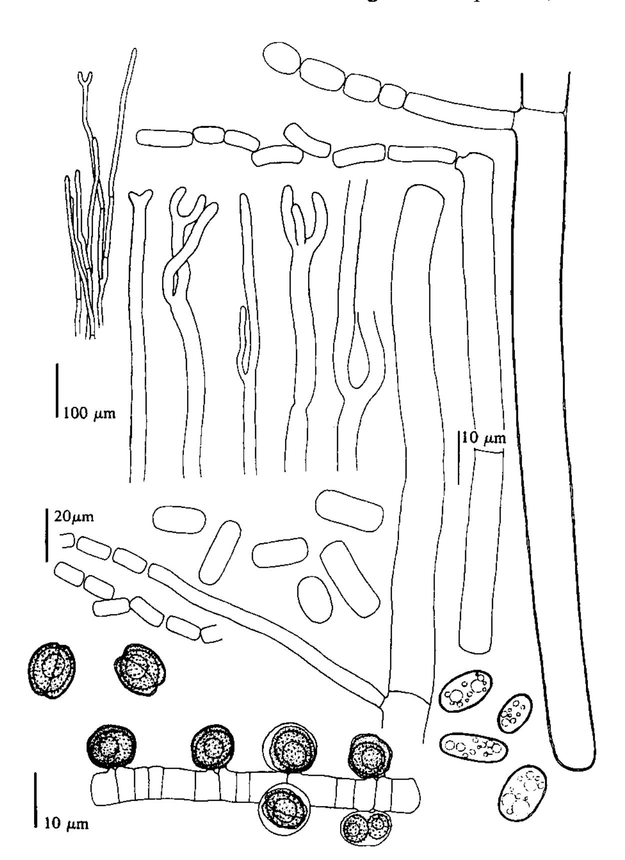


Fig. 92. *G. geotrichum*, CBS 772.71, MEYA, 22°C, 2 weeks. Hyphal tips with di- and trichotomous branching. Mature main branches and disarticulating lateral branches from various cultures; asci with ascospores.

Fermentation:

Glucose	v	Maltose	–
Galactose	v	Lactose	–
Sucrose	–	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	v
Arbutin	–		

Co-Q: Not determined.
Mol% G + C: 42.9–45.2, 11 strains (T_m: de Hoog et al. 1986); 40.7–42.3, 4 strains (BD: Guého et al. 1985).

Origin of the strains studied: CBS 110.12, probably authentic for *Oidium humi*; CBS 122.22, authentic for *Oospora fragrans* var. *minuta*, from banana, Berkhout; CBS 114.23, authentic for *Oidium nubilum*, Berkhout; CBS 116.23, authentic for *Oospora lactis* var. *parasitica*, from rotten tomato, Pritchard and Porte, Maryland; CBS 149.26, authentic for *Oidium asteroides*, Castellani; CBS 178.30, type strain of *Oospora lactis* var. *exuberans*, from *Populus* sap, Stautz; CBS 180.33, authentic for *Geotrichum matalense* var. *chapmanii*; CBS 182.33, type strain of *Geotrichum javanense*, from yoghurt, Verona; CBS 193.34, type strain of *Geotrichum versiforme*, Moore; CBS 195.35, authentic for *Geotrichum matalense*, Castellani; CBS 178.53, type strain of *Endomyces lactis*, Windisch; CBS 772.71 (ATCC 22600), from soil, Puerto Rico, Butler; CBS 557.83, type strain of *Geotrichum novakii*, from prune, Egypt, Zsolt; CBS 626.83, type strain of *Geotrichum pseudocandidum*, from stomach of elk, Paris, Saëz; CBS 279.84, type strain of *Geotrichum redaellii*, Saëz; additional strains isolated from milk (1), *Hyacinthus* bulb (1), tomato (1), human nail (1), light oil (2), human tongue (1), grain (1), cheese (2), soil (2), paper pulp (1), red beet (1) and insect (1).

Complementary mating types: Mating type *a*: CBS 357.86, CBS 774.71 (tester strain), CBS 557.83, CBS 606.85. Mating type *α*: CBS 267.79, CBS 180.33, CBS 775.71 (tester strain), CBS 626.83, CBS 607.85.

Type strain: CBS 772.71, type strain of *Galactomyces geotrichum* and neotype strain of *Geotrichum candidum.*

Comments: *Galactomyces geotrichum*, better known under the anamorph name *Geotrichum candidum,* is common in all kinds of moist substrates, particularly in citrus fruits and tomatoes, and mainly as a rotter of ripe fruits. The species may occur in a relatively early stage of fruit development and hence it has been presumed to be a weak pathogen. In other nutrient-rich substrates it unambiguously occurs as a saprophyte, e.g., in liquid pig's fodder and in lipid-containing materials such as milk and cheese. Thirdly, *G. geotrichum* is occasionally associated with human pulmoneous disease. Probably deep, saprophytic colonization is of concern rather than pathogenicity.

The species is reported to be heterothallic. Crossed strains are fertilized and subsequently fertile hyphae develop which locally become profusely septate with cells often wider than long. On both sides of the septa gametangia develop which fuse and give rise to subglobose asci.

De Hoog et al. (1986) and Smith et al. (1995c), using DNA/DNA reassociation techniques found several groups within the species separated by low or intermediate relatedness. The two tester strains showed intermediate reassociation with remaining groups, which are therefore interpreted as varieties.

Geotrichum candidum is morphologically recognized by its rapid expansion growth, very wide main branches and often dichotomously branched ends of expanding hyphae; in contrast to the anamorphs of *Galactomyces reessii* and *G. citri-aurantii,* it is able to grow on media without vitamins.

33.3. *Galactomyces reessii* (van der Walt) Redhead & Malloch (1977)

Synonyms:
Endomyces reessii van der Walt (1959c)
Dipodascus reessii (van der Walt) von Arx (1977a)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 65 mm in diameter, flat, white, dry and cottony. Expanding hyphae are submerged, and without dichotomous branching. Main branches are 6–9 µm wide, with lateral branches 4–6 µm wide, which soon disarticulate into rectangular arthroconidia (3.5–4.5)×(6–15) µm.

Formation of ascospores: Hyphae with local, profuse septation develop gametangia on opposite sides of septa. Asci are subspherical, (7–10)×(6–8) µm, and contain one ascospore. Ascospores are broadly ellipsoidal, (5–6)×(6–7) µm, pale golden-brown, finely warted, with irregular, locally inflated exosporium, and often with a hyaline median furrow (Fig. 93). The species is homothallic.

Fermentation:

Glucose	v	Maltose	–
Galactose	–	Lactose	–
Sucrose	–	Raffinose	–

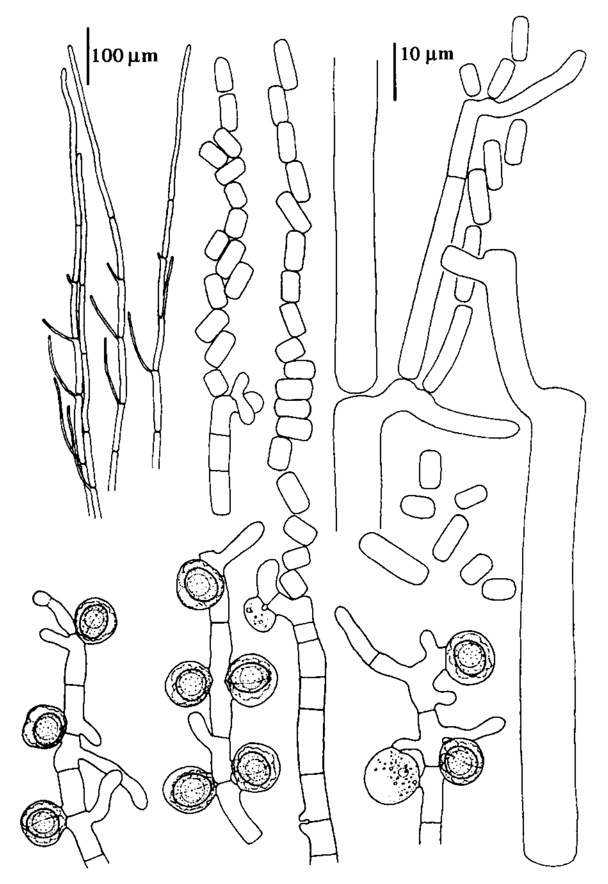


Fig. 93. *G. reessii*, CBS 179.60, MEYA, 22°C, 2 weeks. Branching pattern, disarticulating hyphae and asci with ascospores.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	v
Arbutin	–		

Co-Q: Not determined.

Mol% G+C: 34.5, CBS 179.60 (T_m: Guého 1979); 36.9, CBS 179.60 (BD: Guého et al. 1985).

Origin of the strains studied: CBS 179.60, from water-rotted *Hibiscus*; from fodder (2).

Type strain: CBS 179.60.

Comments: The species is close to *Galactomyces geotrichum* but differentiated by having a smaller micromorphology; the hyphae are less than 9 μm wide and are without dichotomous branching. *G. geotrichum* mostly shows weak fermentation of glucose, and usually growth with D-mannitol, D-glucono-δ-lactone and on media without vitamins. The two species are clearly different in their nuclear DNA G + C content.

Comments on the genus

Kurtzman and Robnett (1995) demonstrated from comparisons of nucleotide sequence divergence in the 5′ end of large subunit rDNA that *Galactomyces* appears congeneric with *Dipodascus*.

34. *Hanseniaspora* Zikes

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is by bipolar budding in basipetal succession on a narrow base. Cells are apiculate, ovoidal to long-ovoidal or elongate. Pseudohyphae are absent or present, usually rudimentary and rarely well-developed. Vegetative cells are diploid.

Asci arise without conjugation. Ascospores are 1–4 per ascus, hat- or helmet-shaped, or spheroidal with warts only, or smooth or warty with an equatorial ledge. Asci are persistent or deliquescent.

Glucose is fermented. Nitrate is not assimilated. Inositol and pantothenate are required for growth. Acetic acid is not produced. Diazonium blue B reaction is negative.

Type species

Hanseniaspora valbyensis Klöcker

Species accepted

1. *Hanseniaspora guilliermondii* Pijper (1928)
2. *Hanseniaspora occidentalis* M.Th. Smith (1974)
3. *Hanseniaspora osmophila* (Niehaus) Phaff, M.W. Miller & Shifrine ex M.Th. Smith (1984)
4. *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak & Phaff ex M.Th. Smith (1984)
5. *Hanseniaspora valbyensis* Klöcker (1912)
6. *Hanseniaspora vineae* van der Walt & Tscheuschner (1957)

Key to species

See Table 25. A key to all described species based entirely on physiological reactions is given under the genus *Kloeckera*.

1.	a Ascospores hat-shaped → 2			
	b Ascospores spheroidal → 3			
2(1).	a Growth at 37°C	....	*H. guilliermondii*:	p. 215
	b Absence of growth at 37°C	....	*H. valbyensis*:	p. 218
3(1).	a Maltose assimilated → 4			
	b Maltose not assimilated → 5			
4(3).	a Growth at 34°C	....	*H. vineae*:	p. 219
	b Absence of growth at 34°C	....	*H. osmophila*:	p. 216
5(3).	a Sucrose assimilated	....	*H. occidentalis*:	p. 215
	b Sucrose not assimilated	....	*H. uvarum*:	p. 217

Table 25
Key characters of species in the genus *Hanseniaspora*

Species	Assimilation		Growth		Ascospores	
	Sucrose	Maltose	at 34°C	at 37°C	Shape	Number[a]
Hanseniaspora valbyensis	–	–	–	–	hat	1–4(2)
H. guilliermondii	–	–	+	+	hat	1–4(4)
H. uvarum	–	–	n	–	spherical, warty + equatorial ledge	1–2
H. occidentalis	+	–	n	–	spherical, smooth + equatorial ledge	1–2
H. osmophila	v	+	–	–	spherical, warty	1–2
H. vineae	v	+	+	–	spherical, warty	1–2

[a] The numbers in parentheses refer to the number of spores per ascus most frequently observed.

Systematic discussion of the species

34.1. *Hanseniaspora guilliermondii* Pijper (1928)

Anamorph: *Kloeckera apis* Lavie ex M.Th. Smith, Simione & S.A. Meyer

Synonyms:

Hanseniaspora melligeri Lodder (1932)
Hanseniaspora apuliensis Castelli (1948)
Kloeckera apiculata (Reess) Janke var. *apis* Lavie (1954)
Kloeckera apis Lavie ex M.Th. Smith, Simione & S.A. Meyer (1977)

Growth in glucose–yeast extract–peptone water: After 2 days at 25°C, the cells are apiculate, ovoidal or elongate, (2.2–5.8)×(4.5–10.2) µm, or occasionally longer, and single or in pairs. Reproduction is by bipolar budding. A sediment is present. After one month a thin ring is formed.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is white to cream-colored, smooth, glossy, and slightly raised at the center.

Dalmau plate cultures on potato agar: Pseudomycelium is rudimentary or absent.

Formation of ascospores: One to four, mostly four, hat- to helmet-shaped ascospores, which are normally released at maturity from the ascus (Fig. 94), often aggregate after liberation.

Abundant sporulation is usually observed on 5% Difco malt extract agar and on potato dextrose agar at low temperature (15°C) after 7 or more days.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
0.01% Cycloheximide	+	Growth at 37°C	+

Co-Q: 6 (Yamada et al. 1976a, Billon-Grand 1987).

Mol% G+C: 32.9–34.2, 11 strains, including CBS 95, CBS 465, CBS 466, CBS 1972, CBS 2574, CBS 2591, CBS 5060 (T_m: Meyer et al. 1978).

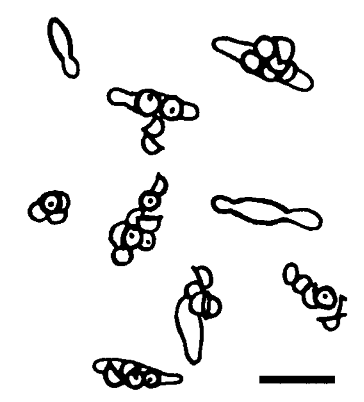


Fig. 94. *H. guilliermondii.* Asci with ascospores on 5% malt extract agar after 7 days at 25°C. Bar = 10 µm (from H.J. Phaff 1970a).

Origin of the strains studied: CBS 465, diseased nail, Pijper; CBS 95, fermenting bottled tomatoes, van Rhee; CBS 466, dates, Chodat, type strain of *H. melligeri*; CBS 1972, grape juice, Castelli, authentic strain of *H. apuliensis*; CBS 2567, grape must, Capriotti; CBS 2574, grape juice, Capriotti; CBS 2591, bee trachea, Chauvin, type strain of *Kloeckera apis*; CBS 4378, caecum of baboon, do Carmo-Sousa; CBS 5060, culture contaminant, Phaff; CBS 6619, CBS 6707, unknown, Nakase.

Type strain: CBS 465 (ATCC 10630, NRRL Y-1625), isolated by Pijper.

Comments: The species *H. apuliensis, H. guilliermondii* and *H. melligeri,* which were placed in synonymy with and maintained as synonyms of *H. valbyensis* by Lodder and Kreger-van Rij (1952) and Phaff (1970a), respectively, were separated from *H. valbyensis* by Meyer et al. (1978) on the basis of low DNA homology. A high degree of reassociation was revealed between *H. apuliensis, H. guilliermondii, H. melligeri* and *Kloeckera apis.*

34.2. *Hanseniaspora occidentalis* M.Th. Smith (1974)

Anamorph: *Kloeckera javanica* (Klöcker) Janke

Synonyms:

Pseudosaccharomyces antillarum Klöcker (1912b)
Kloeckera antillarum (Klöcker) Janke (1928)
Hanseniaspora antillarum Kudryavtsev (1960)
Pseudosaccharomyces indicus Klöcker (1912b)
Kloeckera indica (Klöcker) Janke (1928)
Pseudosaccharomyces javanicus Klöcker (1912b)
Kloeckera javanica (Klöcker) Janke (1928)
Pseudosaccharomyces jensenii Klöcker (1912b)
Kloeckera jensenii (Klöcker) Janke (1928)
Pseudosaccharomyces lafarii Klöcker (1912b)
Kloeckera lafarii (Klöcker) Janke (1928)
Kloeckera javanica (Klöcker) Janke var. *lafarii* (Klöcker) M.W. Miller & Phaff (1958)
Pseudosaccharomyces malaiana Klöcker (1912b)
Kloeckera malaiana (Klöcker) Janke (1928)
Pseudosaccharomyces occidentalis Klöcker (1912b)
Kloeckera occidentalis (Klöcker) Janke (1928)

Kloeckeraspora occidentalis (M.Th. Smith) Maeda, Y. Yamada & Banno (Yamada et al. 1992e)
Pseudosaccharomyces willi Klöcker (1912b)
Kloeckera willi (Klöcker) Janke (1928)
?*Kloeckera cacaoicola* Ciferri (1931b)

Growth in glucose–yeast extract–peptone water: After 2 days at 25°C, the cells are lemon-shaped, ovoidal or sometimes spheroidal, (1.8–6.2)×(3.0–11.0) µm, and single or in pairs. Sediment is formed. After one month a very thin ring is present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is white to cream-colored, smooth, glossy, and with a raised center and a flat periphery.

Dalmau plate culture on potato agar: Pseudomycelium is generally lacking, but some strains produce a rudimentary or well-developed pseudomycelium.

Formation of ascospores: One or two ascospores are formed per ascus and are spheroidal and smooth with an equatorial ledge. Ascospores are not released from the ascus.

Sporulation occurs on 5% Difco malt extract agar at 25°C after 7 or more days.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 34°C	n
Starch formation	–	Growth at 37°C	–
0.01% Cycloheximide	–		

Co-Q: 6 (Yamada et al. 1976a, Billon-Grand 1987).
Mol% G + C: 34.9–35.9, 10 strains, including CBS 282, CBS 283, CBS 284, CBS 2335, CBS 2578, CBS 2592 (T_m: Meyer et al. 1978).

Origin of the strains studied: CBS 2592, soil, Kufferath, type strain of *Pseudosaccharomyces occidentalis*; CBS 280, soil, Winge, type strain of *Pseudosaccharomyces antillarum*; CBS 282, soil, Kufferath, type strain of *Pseudosaccharomyces javanicus*; CBS 283, soil, NCTC, type strain of *Pseudosaccharomyces jensenii*; CBS 284, soil, NCTC, type strain of *Pseudosaccharomyces lafarii*; CBS 2335, type strain of *Kloeckera indica*; CBS 2569, *Drosophila* sp., Shehata; CBS 2578, soil, NCTC, type strain of *Pseudosaccharomyces willi*; CBS 6623, CBS 6624, unknown, Nakase; CBS 6782, CBS 6783, orange juice, van Grinsven.

Type strain: CBS 2592 (ATCC 32053, NRRL Y-7946) isolated by Klöcker and received through Winge.

Comments: DNA reassociation studies by Meyer et al. (1978) showed *H. occidentalis* and *Kloeckera javanica* to have high base sequence complementarity, thus establishing the teleomorph–anamorph relationship, as suggested by Smith (1974) on the basis of physiological characteristics.

34.3. *Hanseniaspora osmophila* (Niehaus) Phaff, M.W. Miller & Shifrine ex M.Th. Smith (1984)

Anamorph: *Kloeckera corticis* (Klöcker) Janke
Synonyms:
Pseudosaccharomyces corticis Klöcker (1912b)
Kloeckera corticis (Klöcker) Janke (1928)
Pseudosaccharomyces magnus de Rossi (1920)
Kloeckera magna (de Rossi) Janke (1928)
Pseudosaccharomyces santacruzensis Klöcker (1912b)
Kloeckera santacruzensis (Klöcker) Janke (1928)
?*Kloeckera domingensis* Ciferri (1930a)
Kloeckeraspora osmophila Niehaus (1932)
Hanseniaspora osmophila (Niehaus) Phaff, M.W. Miller & Shifrine (1956) nom. inval.

Growth in glucose–yeast extract–peptone water: After 2 days at 25°C, the cells are lemon-shaped, ovoidal or long-ovoidal, (3.5–6.0)×(7.2–18.2) µm, and single or in pairs. Sediment is present. After one month, a thin ring is formed.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is white to cream-colored, smooth and glossy; the center is raised and the periphery is flat.

Dalmau plate cultures on potato agar: Branched pseudomycelium is formed.

Formation of ascospores: One to two ascospores are formed per ascus and are spheroidal and warty. They are not released from the ascus.

Sporulation occurs on 5% Difco malt extract agar at 25°C after 7 or more days.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	v	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	0.01% Cycloheximide	–
Starch formation	–	Growth at 34°C	–

Co-Q: 6 (Yamada et al. 1976a, Billon-Grand 1987).
Mol% G+C: 39.8–40.5, 7 strains, including CBS 105, CBS 106, CBS 313, CBS 1999 (T_m: Meyer et al. 1978).

Origin of the strains studied: CBS 313, Riesling grapes, Niehaus; CBS 105 (ATCC 10640, NRRL Y-1611), grapes, Castelli, type strain of *Pseudosaccharomyces magnus*; CBS 106 (ATCC 10635, NRRL Y-1381) tree bark, NCTC[1], type strain of *Pseudosaccharomyces corticis*; CBS 1999, soil, CLSMR[2], type strain of *Pseudosaccharomyces santacruzensis*; CBS 4266, cider, Beech; CBS 6554 (ATCC 20111), unknown, Takeda Chem. Ind. Ltd.; CBS 6622, unknown, IFO; CBS 6704, unknown, Nakase.

Type strain: CBS 313, isolated by Niehaus.

34.4. *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak & Phaff ex M.Th. Smith (1984)

Anamorph: *Kloeckera apiculata* (Reess emend. Klöcker) Janke
Synonyms:
Saccharomyces apiculatus Reess (1870)
Pseudosaccharomyces apiculatus (Reess) Klöcker (1912b)
Kloeckera apiculata (Reess emend. Klöcker) Janke (1928)
Hanseniaspora apiculata Kudryavtsev (1960)
Pseudosaccharomyces austriacus Klöcker (1912b)
Kloeckera austriaca (Klöcker) Janke (1928)
Pseudosaccharomyces germanicus Klöcker (1912b)
Kloeckera germanica (Klöcker) Janke (1928)
Pseudosaccharomyces muelleri Klöcker (1912b)
Kloeckera muelleri (Klöcker) Janke (1928)
Kloeckeraspora uvarum Niehaus (1932)
Hanseniaspora uvarum (Niehaus) Shehata, Mrak & Phaff (1955) nom. inval.
Kloeckera brevis Lodder (1934)
Kloeckera lindneri (Klöcker) Janke var. *pelliculosa* Lodder (1934)
Kloeckera brevis Lodder var. *rohrbachense* von Szilvinyi & Kaulich (1948) nom. inval.
Kloeckera lodderi van Uden & Assis-Lopes (1953a)

Growth in glucose–yeast extract–peptone water: After 2 days at 25°C, the cells are apiculate, spheroidal to ovoidal, elongate, (1.5–5.0)×(2.5–11.5) μm, and single or in pairs. Sediment is present. After one month, a very thin ring is formed.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is white to creamy, smooth, glossy, and slightly raised at the center.

Dalmau plate cultures on potato agar: A rudimentary branched pseudomycelium may be present or absent.

Formation of ascospores: One to two warty, spheroidal ascospores are formed per ascus and have an equatorial or subequatorial ledge (Fig. 95). Both warts and ledge may be inconspicuous under the light microscope. Ascospores are not released from the ascus.

Sporulation occurs on 5% Difco malt extract agar after 4 days or more at 25°C.

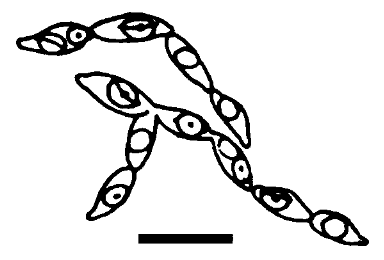


Fig. 95. *H. uvarum.* Asci with ascospores on 5% malt extract agar after 7 days at 25°C. Bar = 10 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

[1] NCTC, National Collection of Type Cultures, U.K.
[2] CLSMR, The former Central Laboratory of the South Manchurian Railroad.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	0.01% Cycloheximide	+
Starch formation	–	Growth at 37°C	–

Co-Q: 6 (Yamada et al. 1976a, Billon-Grand 1987).

Mol% G+C: 32.7–35.1, 20 strains, including CBS 104, CBS 279, CBS 286, CBS 287, CBS 314, CBS 2579, CBS 2580, CBS 2585, CBS 2587 (T_m: Meyer et al. 1978).

Origin of the strains studied: CBS 314 (ATCC 32369, NRRL Y-1614), muscat grapes, Niehaus; CBS 104 (ATCC 32856), unknown, Winge, type strain of *Pseudosaccharomyces apiculatus*; CBS 279, unknown, CLSMR, type strain of *Kloeckera brevis*; CBS 286 (ATCC 10639), soil, NCTC, type strain of *Pseudosaccharomyces malaianus* and *Kloeckera lindneri* var. *pelliculosa*; CBS 287, soil, Winge, type strain of *Pseudosaccharomyces muelleri*; CBS 2579, soil, NCTC, type strain of *Pseudosaccharomyces austriacus*; CBS 2580, soil, Kufferath, type strain of *Pseudosaccharomyces germanicus*; CBS 2585, baker's yeast, van Uden, type strain of *Kloeckera lodderi*; CBS 2587, fruit must, von Szilvinyi, authentic strain of *Kloeckera brevis* var. *rohrbachense*; and 22 additional strains isolated from grapes (1), soil (1), *Drosophila* (2), seawater (4), cacao (1), cucumber brine (1), caterpillar (1), cider (1), throat (1), unknown (4), fruit must (2), fresh water (3).

Type strain: CBS 314, isolated by Niehaus.

34.5. *Hanseniaspora valbyensis* Klöcker (1912b)

Anamorph: *Kloeckera japonica* Saito & Ohtani

Synonyms:

Endomyces valbyensis (Klöcker) Zender (1925b)

Kloeckera japonica Saito & Ohtani (1931)

Kloeckera corticis (Klöcker) Janke var. *pulquensis* Ulloa & Herrera (1973)

Growth in glucose–yeast extract–peptone water: After 2 days at 25°C, the cells are apiculate, spheroidal to ovoidal or elongate, (2.0–5.5)×(3.0–10.2) µm, and single or in pairs (Fig. 96). Sediment is present. After one month a thin ring may be formed.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is white to cream-colored, smooth, glossy, and slightly raised in the center.



Fig. 96. *H. valbyensis*. Budding cells in malt extract after 3 days at 25°C. Bar = 10 µm (from Lodder and Kreger-van Rij 1952).

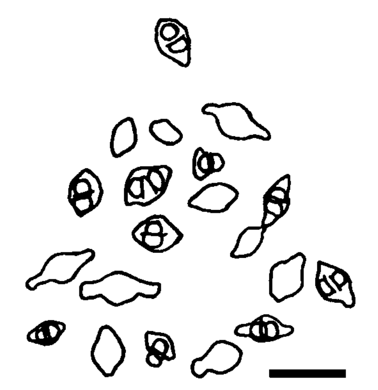


Fig. 97. *H. valbyensis*, CBS 6618. Asci with ascospores on 5% malt extract agar after 7 days at 25°C. Bar = 10 µm.

Dalmau plate cultures on potato agar: A rudimentary branched pseudomycelium may be present or absent.

Formation of ascospores: Two to four, but mostly two, hat- to helmet-shaped ascospores are formed per ascus and they are usually released at maturity (Fig. 97). Liberated ascospores often aggregate.

Ascospores were observed on 5% Difco malt extract agar and potato agar after 7 or more days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	0.01% Cycloheximide	+
Starch formation	–	Growth at 34°C	–

Co-Q: 6 (Yamada et al. 1976a, Billon-Grand 1987).

Mol% G+C: 28.8–30.0, 10 strains, including CBS 281, CBS 311, CBS 479, CBS 6558 (T_m: Meyer et al. 1978).

Origin of the strains studied: CBS 479, soil, Klöcker; CBS 281 (NRRL Y-1382), tree exudate, Inst. Brew. Coll., Hiroshima, type strain of *Kloeckera japonica*; CBS 311,

beer, de Graaf; CBS 480, Winge; CBS 481 (ATCC 2108), unknown, Miller; CBS 2590, draught beer, Johnsonn; CBS 6558 (NRRL Y-7575), pulque, Kurtzman, type strain of *Kloeckera corticis* var. *pulquensis*; CBS 6618, tomato, Nakase; NCYC 468, spoiled beer, Hemmons; NCYC 766, unknown, Davenport.

Type strain: CBS 479, isolated by Klöcker.

34.6. *Hanseniaspora vineae* van der Walt & Tscheuschner (1957b)

Anamorph: *Kloeckera africana* (Klöcker) Janke

Synonyms:

Pseudosaccharomyces africanus Klöcker (1912b)

Kloeckera africana (Klöcker) Janke (1928)

Vanderwaltia vineae (van der Walt & Tscheuschner) Novák & Zsolt (1961)

Hanseniaspora nodinigri Lachance (1981)

Kloeckeraspora vineae (van der Walt & Tscheuschner) Y. Yamada, Maeda & Banno (1992e)

Growth in glucose–yeast extract–peptone water: After 2 days at 25°C, the cells are apiculate, spheroidal to ovoidal or elongate, (2.0–7.5)×(4.0–14.5) µm, and mostly single or sometimes in pairs. Sediment is formed. After one month a very thin ring may be present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is white to cream-colored, smooth and glossy; the center is raised, and the periphery is flat.

Dalmau plate cultures on potato agar: A pseudomycelium is formed, that may be rudimentary or well-developed with or without clusters of blastospores.

Formation of ascospores: One to two spheroidal, warty ascospores are produced per ascus (Fig. 98). Ascospores are not released from the ascus.

Sporulation was observed on 5% Difco malt extract agar and YM agar after 7 days or more at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	v	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

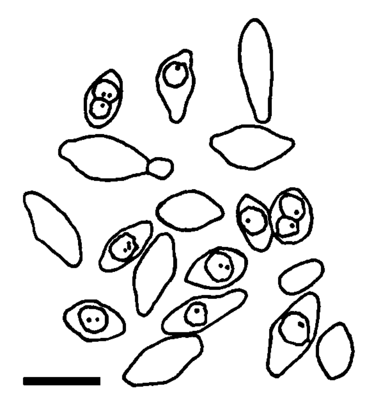


Fig. 98. *H. vineae.* Asci with ascospores on YM after 7 days at 25°C. Bar = 10 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 34°C	+
Starch formation	–	Growth at 37°C	–
0.01% Cycloheximide	+		

Co-Q: 6 (Yamada et al. 1976a, Billon-Grand 1987).

Mol% G+C: 38.8–40.7, 9 strains, including CBS 277, CBS 2171, CBS 6555 (T_m: Meyer et al. 1978); 39.6, CBS 8031 (T_m: Smith and Poot 1985).

Origin of the strain studied: CBS 2171 (ATCC 58436), vinyard soil, van der Walt; CBS 277 (ATCC 24232) soil, Klöcker, type strain of *Pseudosaccharomyces africanus*; CBS 2568, *Drosophila persimilis*, Phaff; CBS 2827, soil, Capriotti; CBS 5068, unknown, Verona; CBS 6555 (ATCC 20109), unknown, Takeda Chem. Ind.; CBS 6706, unknown, Nakase; CBS 8031 (ATCC 46412), black knot gall *Dibotryon morbosum* on *Prunus virginiana*, Lachance, type strain of *H. nodinigri*; ATCC 10632, sour *Calimyrma* fig; ATCC 16512, unknown, Kyowa Ferm. Ind. Co., Ltd.

Type strain: CBS 2171, isolated by van der Walt, South Africa.

Comments: *Hanseniaspora vineae* was placed in synonymy with *H. osmophila* by Miller and Phaff (1958) and maintained as a synonym by Phaff (1970a). However, Meyer et al. (1978) separated the two species since the average DNA reassociation value was 48%. In view of more recent interpretations of DNA relatedness (Kurtzman and Phaff 1987), these values would suggest these two taxa to be sibling species or possible varieties of the same species. The conspecificity of *H. nodinigri* and *H. vineae* was shown by DNA homology studies (Smith and Poot 1985).

Comments on the genus

Miller and Phaff (1958) published a detailed study of the history, nomenclature, physiology, morphology and life cycle of all *Hanseniaspora* species. Antigenic analyses of some *Kloeckera* and *Hanseniaspora* species were carried out by Tsuchiya et al. (1966) and Tsuchiya and Imai (1968). In the former study, 3 serological groups were found, viz. *K. javanica, K. africana* and *K. apiculata*, and the relationship of *K. apiculata* with *H. valbyensis*

was recognized. In addition, *K. javanica* appeared to be a hybrid species of *K. apiculata* and *K. africana*. In the latter study, the authors concluded that (1) *K. apiculata* was the anamorph of *H. uvarum* and *H. guilliermondii*; and (2) *K. africana* was the anamorph of *H. osmophila* and *H. vineae*.

A comparative study on the ultrastructure of the different types of ascospores in *Hanseniaspora* species was performed by Kreger-van Rij and Ahearn (1968) and by Kreger-van Rij (1977b). These studies showed the presence of three types of ascospores: (a) hat-shaped in *H. guilliermondii* and *H. valbyensis*; (b) spheroidal with an equatorial or sub-equatorial ledge, and smooth or warty in *H. occidentalis* and *H. uvarum*; (c) spheroidal with warts in *H. osmophila* and *H. vineae*. Spencer and Gorin (1968) carried out PMR spectrometric studies of mannans isolated from species of the genera *Hanseniaspora* and *Kloeckera*. Yamada et al. (1976a) studied the coenzyme Q systems in apiculate yeast genera, namely *Hanseniaspora, Kloeckera, Nadsonia, Saccharomycodes* and *Wickerhamia*. The Co Q-6 system was found in all species analyzed except for *Wickerhamia*, which had a Co Q-9 system. The authors discussed their results in relation to other criteria, such as PMR spectra of cell wall polysaccharides, DNA base composition and serological characteristics. Billon-Grand (1987) investigated the occurrence of minor components of coenzyme Q systems in *Hanseniaspora* and *Kloeckera*. In addition to the major component Co Q-6, this author found Co Q-7 as a minor component in *H. uvarum, H. valbyensis* and *H. nodinigri*. Fiol and Billon-Grand (1978a) examined the production of intracellular oxidases, nitrite and nitrate reductases in some species of *Hanseniaspora* and *Kloeckera* and discussed the taxonomic relationships between the two genera.

The significance of DNA base composition in the classification of *Hanseniaspora* was studied by Nakase and Komagata (1970a) and Meyer et al. (1978). The latter authors established the status of various *Hanseniaspora* species by DNA–DNA reassociation experiments and correlated *Hanseniaspora* teleomorphs with *Kloeckera* anamorphs. Yamada et al. (1992c) estimated the phylogenetic relationships of the teleomorphic apiculate yeast genera *Hanseniaspora, Nadsonia* and *Saccharomycodes* on the basis of partial rRNA sequences. Their data demonstrated the three genera to be distinct from each other. However, the six species of the genus *Hanseniaspora* were divided into two clusters, one cluster consisted of *H. guilliermondii, H. uvarum* and *H. valbyensis,* the second cluster consisted of *H. occidentalis, H. osmophila* and *H. vineae.* Yamada et al. (1992c) considered the two clusters as genera and reinstated the genus *Kloeckeraspora* to accommodate the latter three species, which are characterized by spheroidal, warty ascospores. This genus, introduced by Niehaus (1932) to accommodate apiculate species producing spheroidal ascospores, was considered a synonym of *Hanseniaspora* by various authors (Lodder and Kreger-van Rij 1952, Phaff 1970a, Meyer et al. 1978, Smith 1984).

A second phylogenetic study on *Hanseniaspora* and possible related genera (Boekhout et al. 1994) based on partial 26S rDNA nucleotide sequences, demonstrated that the genus *Hanseniaspora* is monophyletic and could be divided into the same subgroups as in the study of Yamada et al. (1992c,e). However, Boekhout et al. argued for maintenance of all species in *Hanseniaspora* on the basis of both the heterogeneous distribution of phenetic properties among species of this genus as well as the low statistical support in the 26S rDNA tree for separation of the two subgroups.

35. *Issatchenkia* Kudryavtsev emend. Kurtzman, Smiley & Johnson

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ellipsoidal or elongate, and pseudohyphae are present.

Asci are unconjugated when formed from diploid cells or may be conjugated if formed by the pairing of complementary mating types. Asci are persistent and form 1–4 roughened spheroidal ascospores. The protuberances causing ascospore roughness may not be visible under the light microscope.

Glucose is fermented. Nitrate is not assimilated. Early formation of pellicles on liquid media. Diazonium blue B reaction is negative.

Type species

Issatchenkia orientalis Kudryavtsev

Species accepted

1. *Issatchenkia occidentalis* Kurtzman, Smiley & Johnson (1980)
2. *Issatchenkia orientalis* Kudryavtsev (1960)
3. *Issatchenkia scutulata* (Phaff, M.W. Miller & Miranda) Kurtzman, Smiley & Johnson (1980)
   a. *Issatchenkia scutulata* (Phaff, M.W. Miller & Miranda) Kurtzman, Smiley & Johnson var. *scutulata* (1980)
   b. *Issatchenkia scutulata* var. *exigua* (Phaff, M.W. Miller & Miranda) Kurtzman, Smiley & Johnson (1980)
4. *Issatchenkia terricola* (van der Walt) Kurtzman, Smiley & Johnson (1980)

Key to species

See Table 26.

1.	a Growth in vitamin-free medium → 2		
	b Growth absent in vitamin-free medium	*I. terricola*:	p. 225
2(1).	a D-Glucosamine assimilated → 3		
	b D-Glucosamine not assimilated → 4		
3(2).	a Growth at 40°C	*I. orientalis*:	p. 222
	b Growth absent at 40°C	*I. occidentalis*:	p. 221
4(2).	a Growth in osmotic medium (10% sodium chloride/5% glucose in yeast nitrogen base)	*I. scutulata* var. *scutulata*:	p. 224
	b Growth absent in osmotic medium	*I. scutulata* var. *exigua*:	p. 224

Table 26
Key characters of species in the genus *Issatchenkia*

Species	Fermentation of glucose	Assimilation			Growth			
		D-Glucosamine	Glycerol	Citrate	Vitamin-free medium	Osmotic medium[a]	37°C	40°C
Issatchenkia occidentalis	+	+	+	–	+	+	+	–
I. orientalis	+	+	+	+/w	+	+	+	+
I. scutulata var. *scutulata*	+	–	+/s	–	+	+	–	–
I. scutulata var. *exigua*	w/s	–	–	–	+	–	–	–
I. terricola	+/w	–	+	w/–	–	–	v	–

[a] 10% sodium chloride/5% glucose in yeast nitrogen base.

Systematic discussion of the species

35.1. *Issatchenkia occidentalis* Kurtzman, Smiley & Johnson (1980b)

Anamorph: *Candida sorbosa* Hedrick & Burke ex van Uden & H.R. Buckley

Synonyms:

Candida sorbosa Hedrick & Burke (1951) nom. nud.

Candida sorbosa Hedrick & Burke ex van Uden & H.R. Buckley (1970)

Candida soli Okuma & S. Goto (Goto et al. 1987b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2.2–4.3)×(3.2–9.3) µm, and single or in pairs. Growth is butyrous and light cream colored.

Growth on the surface of assimilation media: Dry climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and moderately well-branched pseudohyphae. Aerobic

growth is white to tannish-white, dull, butyrous, striated, and slightly raised with a flattened center. The margin is smooth, finely serrate, or with small lobes. Colonies are fringed with pseudohyphae. A faintly acidic odor is present.

Formation of ascospores: Asci are unconjugated, persistent, and form one or two roughened ascospores (Fig. 99). Electron microscopy may be required to discern the protuberances on spore walls (Kurtzman and Smiley 1974, Kurtzman et al. 1980b). Isolation of single spores demonstrated this species to be heterothallic (Kurtzman and Smiley 1976). Following conjugation of complementary mating types, zygotes usually give rise to a succession of diploid cells that sporulate; consequently, conjugated asci are rarely, if ever, observed.

Ascospores were observed on autoclaved cucumber and carrot wedges after 3–6 weeks at 25°C. Sporulation is usually sparse.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+/w
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+/w
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+	Growth at 40°C	–

Co-Q: 7 (CBS 5459, Y. Yamada, personal communication).
Mol% G+C: 40.9–41.1, two strains, CBS 5459, CBS 1910 (BD: Kurtzman et al. 1980b).

Origin of the strains studied: NRRL Y-7552 (CBS 5459), spoiled figs?, Phaff, U.S.A., originally identified as a citrate-negative strain of *Pichia kudriavzevii* (Kreger-van Rij 1970c); NRRL Y-7767 (CBS 1910), type strain of *Candida sorbosa* from feces of a fly *(Drosophila crucigera),* Hedrick, Hawaii, U.S.A.; NRRL Y-7768 (CBS 2107), 'tea fungus', Boedijn, Indonesia.

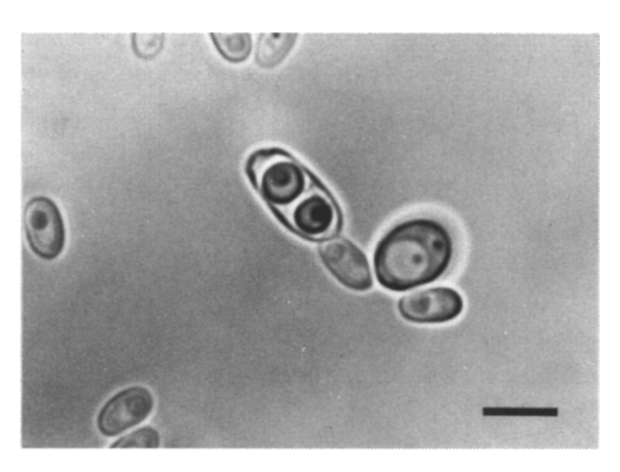


Fig. 99. *I. occidentalis,* CBS 5459. Unconjugated ascus with ascospores, after 1 month on an autoclaved cucumber wedge at 25°C. Bar = 5 μm.

Complementary mating types: NRRL Y-7552-61 (CBS 6887) and NRRL Y-7552-62 (CBS 6888), both single-ascospore isolates from the type strain.

Type strain: CBS 5459 (NRRL Y-7552), a diploid strain received from Phaff, U.S.A.

Comments: *I. occidentalis* was initially classified as a strain of *I. orientalis*, but Kreger-van Rij (1970c) noted that this strain (CBS 5459) differed from the others in being sorbose-positive and citrate-negative. Kurtzman and Smiley (1976) demonstrated this strain to be heterothallic and that one of the mating types conjugated with the type strain of *Candida sorbosa* and formed ascospores. The other strains of *I. orientalis* and several strains of *Candida krusei* also conjugated with one of the mating types, but the frequency was quite low and no ascospores formed.

DNA reassociation studies by Kurtzman et al. (1980b) showed *I. occidentalis* and *C. sorbosa* to have 98% base sequence complementarity, thus confirming the teleomorph–anamorph relationship demonstrated by mating tests. The extent of DNA reassociation among strains of *I. orientalis* and *C. krusei* was 93–100%, but when DNA from these strains was compared with that from *I. occidentalis*, only 8% common sequences were detected. Consequently, evidence from both mating tests and DNA studies shows the presence of two taxa, but a close relationship is suggested. The heterothallic diploid type strain of *I. occidentalis* was shown to be heterozygous for L-sorbose assimilation; therefore, this compound is of questionable utility for separating *I. occidentalis* from *I. orientalis*.

35.2. *Issatchenkia orientalis* Kudryavtsev (1960)

Anamorph: *Candida krusei* (Castellani) Berkhout
Synonyms:
Saccharomyces krusei Castellani (1910?)
Endomyces krusei Castellani (1912b)
Monilia krusei (Castellani) Castellani & Chalmers (1913)
Candida krusei (Castellani) Berkhout (1923)

Myceloblastanon krusei (Castellani) Ota (1928)
Geotrichoides krusei (Castellani) Langeron & Talice (1932)
Trichosporon krusei (Castellani) Ciferri & Redaelli (1935)
Mycotoruloides krusei (Castellani) Langeron & Guerra (1935)
?*Enantiothamnus braulti* Pinoy (Brault and Masselot 1911)
?*Blastodendrion braulti* (Pinoy) Langeron & Talice (1932)
Monilia parakrusei Castellani (Castellani and Chalmers 1919)
Myceloblastanon parakrusei (Castellani) Ota (1928)
Castellania parakrusei (Castellani) Dodge (1935)
Candida parakrusei (Castellani) Langeron & Guerra (1938)
Mycoderma chevalieri Guilliermond (1914)
Candida chevalieri (Guilliermond) Westerdijk (in CBS catalogue, 1933)
Mycoderma monosa Anderson (1917)
Mycoderma bordetii Kufferath (1920)
Monilia inexpectata Mazza, Niño & Egües (1930)
Mycocandida inexpectata (Mazza, Niño & Egües) Talice & Mackinnon (1934)
Pseudomonilia inexpectata (Mazza, Niño & Egües) Dodge (1935)
Trichosporon dendriticum Ciferri & Redaelli (1935) nom. nud.
Candida dendritica (Ciferri & Redaelli) Dodge & Moore (1936)
?*Castellania africana* (Macfie) Dodge (1935)
?*Castellania balcanica* (Castellani & Chalmers) Dodge (1935)
Monilia krusoides Castellani (1937a)
Pseudomycoderma miso Mogi (1939) nom. nud.
Candida castellanii van Uden & Assis-Lopes (1953b)
Candida tamarindi Lewis & Johar (1955) nom. nud.
Procandida tamarindii (Lewis & Johar) Novák & Zsolt (1961)
Candida lobata Batista & Silveira (1959c)
Endoblastomyces thermophilus Odincova ex Kudryavtsev (1960)
Candida requinyii Szép & Novák (1963)
Candida soosii Novák (1964)
Pichia orientalis (Kudryavtsev) Kreger-van Rij (1964b) [non *Saccharomyces orientalis* Beijerinck (1898a) nec *Pichia orientalis* (Beijerinck) Guilliermond (1912)]
Pichia kudriavzevii Boidin, Pignal & Besson (1965b)
Candida acidothermophilum Masuda, Kato, Takayama, Kida & Nakanishi (1975)
Candida brassicae Amano, S. Goto & Kagami (1975)
Candida ethanothermophilum Masuda, Kato, Takayama, Kida & Nakanishi (1975)
?*Candida melinii* Diddens & Lodder var. *melobiosica* Nowakowska-Waszczuk & Pietka (1983)
Candida hinoensis Iwasaki & S. Goto (Goto et al. 1987b)
Candida solicola Endo, Okuma & S. Goto (Goto et al. 1987b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (1.3–6.0)×(3.3–14.0) μm, and single or in pairs. Growth is butyrous and light cream colored.

Growth on the surface of assimilation media: Heavy, dry climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and moderately branched pseudohyphae. Aerobic growth is tannish-white, dull to occasionally almost powdery, butyrous, low convex with a flattened center, margin varying from smooth to lobed, and fringed with pseudohyphae. Some cultures have a faintly acidic odor.

Formation of ascospores: Ascospores were not observed in the present study. Kudryavtsev (1960) reported the spores to be spheroidal, smooth under the light microscope, and one per ascus. Asci were persistent. Two of the three strains observed by Kreger-van Rij (1970c) had occasional asci with one to two spheroidal ascospores, but the infrequency of spores prevented their observation under the electron microscope.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+/w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+	Growth at 40°C	+

Co-Q: 7 (Yamada et al. 1973a).

Mol% G+C: 39.7–40.3, CBS 5147, CBS 573 and four other strains (BD: Kurtzman et al. 1980b).

Origin of the strains studied: Isolates originally reported to form ascospores: CBS 5147, fruit juice or berries, Kudryavtsev, Russia; CBS 2911, tea beer, Netherlands; CBS 5590, unknown, Suomalainen, Finland. Isolates received as *Candida krusei*: bronchomycosis (1); vaginitis (1); human feces (1); human urine (1); mastitic cow (2); animal feces (4); fruit flies (*Drosophila* spp.) (5); ginger beer (1); apple cider (1); stored rice (1); stored corn (2); bread (1); fermented foods (3); dairy products (3); industrial fermentation (1); yeast cake (1); tree exudates (2); seawater (1); unknown (15).

Type strain: CBS 5147 (NRRL Y-5396), received from Kudryavtsev, Russia.

Comments: *Candida krusei* is considered to represent the anamorphic form of *I. orientalis* because the type strains, as well as other isolates of both species, showed significant (93–100%) DNA base sequence complementarity (Kurtzman et al. 1980b). *C. requinyii* also showed significant base sequence relatedness with *C. krusei* and *I. orientalis* but not with *I. occidentalis*. The relationship between *I. orientalis* and *I. occidentalis* is discussed in the section on the latter species.

I. orientalis and its anamorph *C. krusei* are isolated

from a wide variety of habitats including humans and animals. Hurley et al. (1987) summarized clinical evidence supporting the concept that *C. krusei* should be considered pathogenic rather than a transient saprophyte.

35.3. *Issatchenkia scutulata* (Phaff, M.W. Miller & Miranda) Kurtzman, Smiley & Johnson (1980b)

This species has two varieties:

***Issatchenkia scutulata* (Phaff, M.W. Miller & Miranda) Kurtzman, Smiley & Johnson var. *scutulata* (1980)**

Synonym:

Pichia scutulata Phaff, M.W. Miller & Miranda var. *scutulata* (1976)

***Issatchenkia scutulata* var. *exigua* (Phaff, M.W. Miller & Miranda) Kurtzman, Smiley & Johnson (1980)**

Synonym:

Pichia scutulata var. *exigua* Phaff, M.W. Miller & Miranda (1976)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2.9–5.5)×(3.5–10.0) µm, and single or in short chains. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Moderate to heavy, dry climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass is composed of moderately branched pseudohyphae. Aerobic growth is tannish-white, butyrous, low convex with a depressed center, and with a finely lobed margin. There is a faintly acidic odor.

Formation of ascospores: Asci are persistent, unconjugated and usually form four (one to four) spheroidal, roughened spores in diamond-shaped tetrads (Fig. 100). The species is heterothallic and four-spored asci give two spores of each mating type. Asci arising from a mixture of complementary mating types are generally unconjugated because of proliferation of diplophase cells before sporulation. Ascospore roughness is visible only under the electron microscope and results from small protuberances originating in the inner spore wall (Kurtzman et al. 1980b).

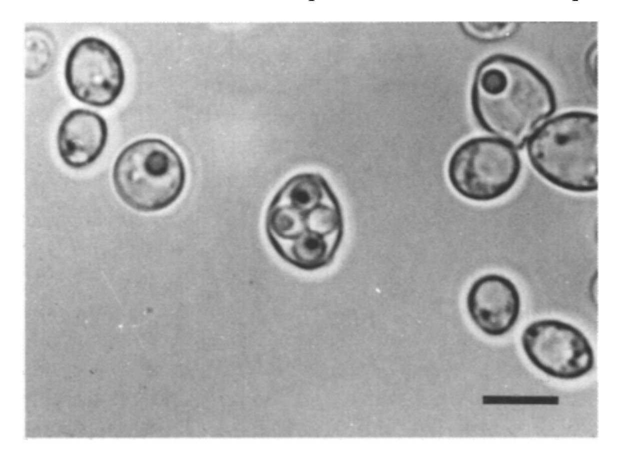


Fig. 100. *I. scutulata* var. *scutulata*, CBS 6670. Unconjugated ascus with ascospores after 2 weeks on YM agar at 25°C. Bar = 5 µm.

Ascospores were observed for both varieties on YM- and 5% Difco malt extract agar after 1–2 weeks at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+/w
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 7 (Y. Yamada, personal communication).

Mol% G+C: 32.6–33.0, three strains of the variety *scutulata*; 32.4–32.6, three strains of the variety *exigua* (BD: Phaff et al. 1976).

Supplementary description of *I. scutulata* var. *exigua*: The variety *exigua* does not assimilate glycerol or grow in osmotic medium (10% NaCl/5% glucose) but does assimilate *N*-acetyl-D-glucosamine, and these differences allow it to be separated from the variety *scutulata* using standard growth tests. Furthermore, glucose fermentation is slow or weak. Morphologically, the varieties are similar. Asci of the variety *exigua* are frequently diamond-shaped, but a linear arrangement of ascospores is not uncommon. Ascospore ultrastructure is similar to that of the variety *scutulata* except that the protuberances seem to arise from the outer layer of the spore wall. The variety *exigua* is also heterothallic.

Origin of the strains belonging to the variety *scutulata*: Slime flux of sandalwood *(Myoporum sandwicense)*, Hawaii, U.S.A. (3).

Complementary mating types: NRRL Y-7663-2 (CBS 6672) and NRRL Y-7663-3 (CBS 6673), both single-ascospore isolates from the type strain.

Type strain: CBS 6670 (UCD-FST 71-102, NRRL Y-7663) received from Phaff.

Origin of the strains belonging to the variety *exigua*: Insect boring in sitka spruce *(Picea sitchensis)*, Washington, U.S.A. (1); slime flux of black cottonwood *(Populus trichocarpa)*, British Columbia, Canada (1); slime flux of willow (*Salix* sp.), British Columbia, Canada (1).

Complementary mating types: NRRL Y-10920-3 (CBS 8067) and NRRL Y-10920-4 (CBS 8068), both obtained as single-ascospore isolates from the type strain.

Type strain: CBS 6836 (UCD-FST 68-979B1, NRRL Y-10920), isolated from an insect boring in sitka spruce, Washington, U.S.A.; received from Phaff.

Comments: Phaff et al. (1976) separated the two varieties because of the physiological differences enumerated above and on the basis of geographically separate habitats. Kurtzman et al. (1980b) determined the extent of nuclear DNA base sequence complementarity between the two varieties to be only 25%, and this would suggest that they are separate species (Price et al. 1978, and references cited therein). However, intervarietal matings gave asci with spores that appeared normal and a small number of the spores proved viable. Sib-matings between these progeny gave viable spores. On the basis of this intervarietal fertility, the variety designations have been maintained.

35.4. *Issatchenkia terricola* (van der Walt) Kurtzman, Smiley & Johnson (1980b)

Synonyms:
Pichia terricola van der Walt (1957)
Saccharomyces terricolus (van der Walt) Novák & Zsolt (1961)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2.4–5.8)×(3.0–14.7) µm, and single or in pairs. Growth is butyrous and tannish-cream in color.

Growth on the surface of assimilation media: Moderate to heavy, dry climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows poorly to moderately branched pseudohyphae. Aerobic growth is white to tannish-white, dull to faintly glistening, butyrous, low convex with a flattened center, and a lobed margin. The odor is faintly acidic.

Formation of ascospores: Asci are unconjugated, persistent and contain one to four, but usually two, spheroidal spores. Ascospores are roughened as a result of protuberances which originate in the outer spore wall, and this roughness can usually be perceived in the light microscope (Fig. 101). Kurtzman and Smiley (1976) demonstrated this species to be heterothallic. During laboratory cultivation, diploid cultures frequently become haploid to the exclusion of one mating type, and the type strain as well as several other cultures are now asporogenous haploids. Asci arising from a mixture of complementary mating types are usually unconjugated because of the proliferation of diploid cells before sporulation.

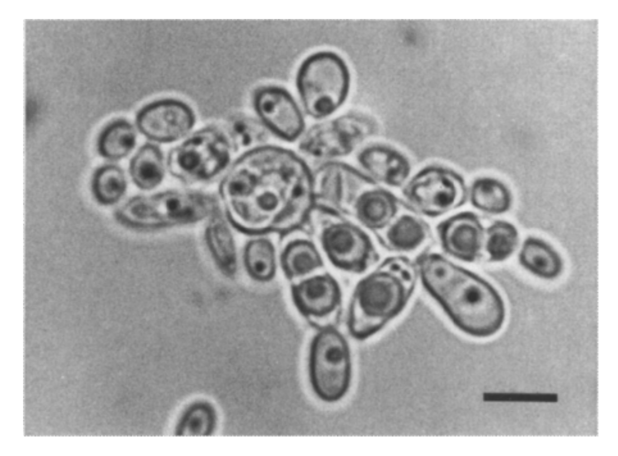


Fig. 101. *I. terricola*, CBS 2617×CBS 6893. Cluster of 2-spored unconjugated asci after 3 weeks on 5% malt extract agar at 25°C. Bar = 5 µm.

Ascospores were observed on YM-, 5% Difco malt extract-, and V8 agar after 1–3 weeks at 25°C.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	w/–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	v	Growth at 40°C	–

Co-Q: 7 (Yamada et al. 1973a).

Mol% G + C: 37.9, 38.2, CBS 2617, NRRL Y-8218 (BD: Kurtzman et al. 1980b).

Origin of the strains studied: Soil, South Africa (1); soil, Italy (1); soil, Louisiana, U.S.A. (2); cherry juice (1); dregs of pressed grapes, Italy (1); sea water (1); spoiled figs, U.S.A. (1); old wine (1).

Complementary mating types: NRRL YB-4310 (CBS 2617) and NRRL Y-7549-25 (CBS 6893).

Type strain: CBS 2617 (NRRL YB-4310), soil isolate received from van der Walt, South Africa.

Comments on the genus

Kudryavtsev (1960) described the monotypic genus *Issatchenkia* on the basis of certain strains isolated from berries and fruit juices. The cultures formed spores that were spheroidal and smooth when observed with the light microscope. Asci were persistent and contained one spore. Kreger-van Rij (1964b) transferred the single species to the genus *Pichia* as *P. orientalis,* but Boidin et al. (1965b) pointed out that the name had been used previously and proposed the new name *P. kudriavzevii.*

Pichia terricola and *P. scutulata* were two other species phenotypically similar to *P. kudriavzevii.* Scanning electron microscopy showed the former two species and *I. occidentalis* to have warty ascospore walls, and in this respect, to differ from other spheroidal-spored species of *Pichia* which have essentially smooth spore walls (Kurtzman and Smiley 1974, Phaff et al. 1976). Despite their similarity in ascospore surface structure to species of *Debaryomyces* and *Torulaspora* (Kurtzman et al. 1975), these three species do not exhibit the cell–bud conjugation typical of *Debaryomyces* or the protuberances on asci that are associated with sporulation in *Torulaspora.* Furthermore, all species now assigned to *Issatchenkia* have Q-7 ubiquinone in the electron transport system while all *Saccharomyces* and *Torulaspora* species have Q-6 ubiquinone. Species of *Debaryomyces* and the remainder of the *Pichia* species with spheroidal spores have Q-9 ubiquinone (Yamada et al. 1973a, 1976b, 1977; Yamada, personal communication). Peterson and Kurtzman (1990) estimated phylogenetic relatedness among *Issatchenkia* spp. from extent of nucleotide substitution in three regions of rRNA. *I. terricola* was the most divergent member of the group.

36. *Kluyveromyces* van der Walt emend. van der Walt

M.A. Lachance

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are ovoidal, ellipsoidal, cylindrical to elongate. Pseudomycelium may be formed. True hyphae are not produced.

Conjugation may or may not precede ascus formation. The ascospores are smooth, reniform, bacilliform, ellipsoidal or spheroidal, tending to agglutinate after liberation. One to four, or in some species, many ascospores are formed per ascus.

Glucose is fermented vigorously. Nitrate is not assimilated. Diazonium blue B reaction is negative.

Type species

Kluyveromyces polysporus van der Walt

Species accepted

1. *Kluyveromyces aestuarii* (Fell) van der Walt (1971)
2. *Kluyveromyces africanus* van der Walt (1956)
3. *Kluyveromyces bacillisporus* Lachance, Phaff & Starmer (1993)
4. *Kluyveromyces blattae* Henninger & Windisch (1976)
5. *Kluyveromyces delphensis* (van der Walt & Tscheuschner) van der Walt (1971)
6. *Kluyveromyces dobzhanskii* (Shehata, Mrak & Phaff) van der Walt (1971)
7. *Kluyveromyces lactis* (Dombrowski) van der Walt (1971)
   a. *Kluyveromyces lactis* (Dombrowski) van der Walt var. *lactis* (1986)
   b. *Kluyveromyces lactis* var. *drosophilarum* (Shehata, Mrak & Phaff) Sidenberg & Lachance (1986)
8. *Kluyveromyces lodderae* (van der Walt & Tscheuschner) van der Walt (1971)
9. *Kluyveromyces marxianus* (E.C. Hansen) van der Walt (1971)
10. *Kluyveromyces phaffii* (van der Walt) van der Walt (1971)
11. *Kluyveromyces polysporus* van der Walt (1956)
12. *Kluyveromyces thermotolerans* (Filippov) Yarrow (1972)
13. *Kluyveromyces waltii* K. Kodama (1974)
14. *Kluyveromyces wickerhamii* (Phaff, M.W. Miller & Shifrine) van der Walt (1971)
15. *Kluyveromyces yarrowii* van der Walt, E. Johannsen, Opperman & Halland (1986)

Key to species

See Tables 27 and 28.

1. a Ethylamine and L-lysine assimilated strongly (groups B and C) → 2
   b Ethylamine and L-lysine not assimilated strongly (group A) → 10

2(1). a Lactose assimilated → 3
   b Lactose not assimilated → 6

3(2). a Growth in the presence of cycloheximide (100 mg/l) → 4
   b Growth absent in the presence of cycloheximide (100 mg/l) .... *K. aestuarii*: p. 229

4(3). a Melezitose assimilated .... *K. lactis* var. *lactis*: p. 233
   b Melezitose not utilized → 5

5(4). a Inulin assimilated .... *K. marxianus*: p. 236
   b Inulin not assimilated .... *K. wickerhamii*: p. 242

6(2). a Growth at 37°C vigorous → 7
   b Growth at 37°C weak or absent → 8

7(6). a Inulin assimilated strongly .... *K. marxianus*: p. 236
   b Inulin utilized weakly or not at all .... *K. lactis* var. *drosophilarum*: p. 234

8(6). a Cellobiose assimilated .... *K. dobzhanskii*: p. 233
   b Cellobiose not assimilated → 9

9(8). a Growth in the presence of cycloheximide (100 mg/l) .... *K. waltii*: p. 241
   b Growth absent in the presence of cycloheximide (100 mg/l) .... *K. thermotolerans*: p. 240

10(1). a 2-Keto-D-gluconate assimilated → 11
   b 2-Keto-D-gluconate not assimilated → 12

11(10). a Galactose assimilated .... *K. blattae*: p. 231
   b Galactose not assimilated .... *K. bacillisporus*: p. 230

12(10). a	Sucrose assimilated → 13		
b	Sucrose not assimilated → 14		
13(12). a	Asci multispored	*K. polysporus*:	p. 239
b	Asci one- to four-spored	*K. lodderae*:	p. 235
14(12). a	Galactose assimilated	*K. delphensis*:	p. 232
b	Galactose not assimilated → 15		
15(14). a	Ascospores spherical, produced only after mixing compatible cultures	*K. yarrowii*:	p. 243
b	Ascospores ellipsoidal to reniform, produced in single cultures → 16		
16(15). a	Asci multispored (1 to 16 or more ascospores)	*K. africanus*:	p. 230
b	Asci one- to four-spored	*K. phaffii*:	p. 239

Table 27
Key characters of *Kluyveromyces* group A species

Species	Assimilation/fermentation[a]														Other[b]					
	Gal	Su/Raf	Tre	Eth	Gly	Glu	Laa	Suc	Cit	2Kg	EtN	Lys	Cad	G50	Cyc	Aaf	37C	SpS[c]	SpN	Sex[d]
Kluyveromyces polysporus	+[e]	+[e]	+/s[e]	s/–	+	+	w/–	w/s	+/s	–	–	–	–	–	–	+	w/–	e/r	100	Hom
K. lodderae	+[e]	+[e]	+[e]	s	+	–	+	w/–	–	–	s/–	–	+/s	–	+	+	–	e/r	4	Hom
K. phaffii	+[e]	–	–	s/–	+	s	–	–	–	–	–	–	–	–	–	+	–	e/r	4	Hom
K. yarrowii	+[e]	–	–	w/s	v	–	–	–	–	–	–	–	–	–	–	+	–	s	4	Het
K. africanus	+/w[e]	–	–	–	w/–	–	–	–	–	–	–	–	–	–	–	w	w/–	e/r	16	Hom
K. blattae	+[e]	–	–	–	w/–	–	–	–	–	+	–	–	–	–	–	–	–	s/e	4–9	Hom
K. bacillisporus	–	w/s	–	–	s	+	–	–	–	+	–	–	–	s	–	+	w	bf	4–8	Hom
K. delphensis	–	–	w/s	s	+	s	w	–	–	–	–	w/s	–	w/s	–	+	+	r	4	Hom

[a] Abbreviations: Gal, galactose; Su/Raf, sucrose and raffinose; Tre, trehalose; Eth, ethanol; Gly, glycerol; Glu, D-gluconic acid; Laa, DL-lactate; Suc, succinate; Cit, citrate; 2Kg, 2-keto-D-gluconate; EtN, ethylamine HCl; Lys, L-lysine; Cad, cadaverine; G50, 50% glucose–yeast extract agar.
[b] Abbreviations: Cyc, growth in cycloheximide 100 mg/l; Aaf, amino acid-free; 37C, growth at 37°C; SpS, spore shape; SpN, spore number; Sex, sexuality.
[c] Abbreviations: bf, bacilliform; e, ellipsoidal; r, reniform; s, spheroidal.
[d] Abbreviations: Hom, homothallic; Het, heterothallic.
[e] Fermented also.

Table 28
Key characters of *Kluyveromyces* groups B and C species

Species	Assimilation/fermentation[a]										Other[b]				
	Gal	αGl	Tre	Lac	In	Ce/Sal	Xyl	Laa	Suc	Cit	G50	Cyc	37C	SpS[c]	Sex[d]
Group B															
Kluyveromyces dobzhanskii	+[e]	+[e]	+	–	–	+	s/–	+/s	+	v	s/–	+	w/–	r	Hom
K. lactis var. *drosophilarum*	+[e]	v[e]	v[e]	–	v[e]	v	v	v	+	v	v	+	+	s/r	Hom
K. lactis var. *lactis*	+[e]	v[e]	+[e]	+[e]	v	+	v	v	+	–	v	+	w/–	s/e	Het
K. marxianus	+/s[e]	–	w/–	v[e]	+[e]	v	+/s	+	+	v	–	+	+	s/r	Hom
K. wickerhamii	+[e]	–	s/–	+	–	+/w	+	v	v	–	–	+	v	r	Hom
K. aestuarii	+[e]	–	w/–	+	–	+	–	+	+	–	–	–	–	s/e	Hom
Group C															
K. thermotolerans	+[e]	+[e]	+[e]	–	s/–[e]	–	–	–	v	–	+	–	v	s	Hom
K. waltii	–	–	–	–	–	–	s	–	–	–	w/s	+	w/–	s	Hom

[a] Abbreviations: Gal, galactose; αGl, maltose/melezitose/α-methyl-D-glucoside; Tre, trehalose; Lac, lactose; In, inulin; Ce/Sal, cellobiose and salicin; Xyl, D-xylose; Laa, DL-lactate; Suc, succinate; Cit, citrate.
[b] Abbreviations: G50, 50% glucose–yeast extract agar; Cyc, growth in cycloheximide 100 mg/l; 37C, growth at 37°C; SpS, spore shape; Sex, sexuality.
[c] Abbreviations: e, ellipsoidal; s, spheroidal; r, reniform.
[d] Abbreviations: Hom, homothallic; Het, heterothallic.
[e] Fermented also.

Systematic discussion of the species

36.1. *Kluyveromyces aestuarii* (Fell) van der Walt (1971)

Synonyms:
Saccharomyces aestuarii Fell (1961)
Kluyveromyces aestuarii (Fell) van der Walt (1965c) nom. inval.
Zygofabospora aestuarii (Fell) Naumov (1987b)

Growth on YM agar: After 3 days at 25°C, the cells are globose to ellipsoidal, (2–6)×(3–7) µm, single, in pairs or in short chains. Growth is butyrous, white to deep ochre because of pulcherrimin production, and glossy or dull.

Growth in glucose–yeast extract broth: A sediment and a thin pellicle are formed.

Dalmau plate culture on corn meal agar: After two weeks a rudimentary pseudomycelium is formed.

Formation of ascospores: Conjugation between cells of the same culture or between bud and parent-cell usually precedes ascus formation. One to four spheroidal to ellipsoidal ascospores with central refringent globules are formed (Fig. 102). The spores are liberated from the ascus soon after formation and tend to agglutinate.

Abundant sporulation occurs after 2–4 days at 17–25°C on most commonly used media. Both strains studied were sporogenous.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	w/–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	w/–	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	+	Cycloheximide 100 mg/l	–
L-Lysine	+	Amino acid-free	+
Ethylamine·HCl	+	Growth at 37°C	–
50% Glucose	–		

Co-Q: Not determined.

Mol% G+C: 39.8–39.9, 2 strains, CBS 4438 and CBS 4904 (BD: Fuson et al. 1987).

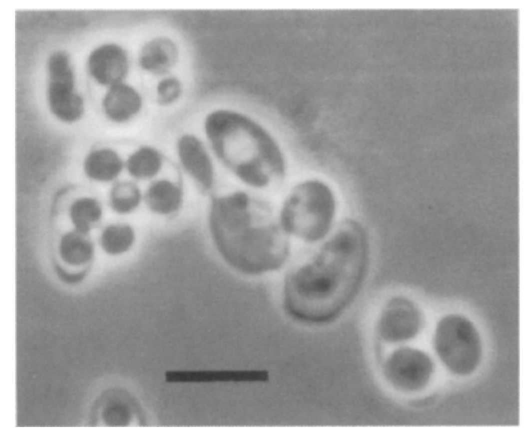


Fig. 102. Asci of *K. aestuarii*, CBS 4438, after 2 weeks on YM agar. Bar = 5 µm.

Origin of the strains studied: CBS 4438, marine mud, Biscayne Bay, Fell, U.S.A.; CBS 4904, sea water, Torres Strait, van Uden, Portugal.

Type strain: CBS 4438, isolated by Fell from marine mud.

Comments: *K. aestuarii* is presumed to be a marine species, although special physiological adaptations to saline environments have not been fully documented. Growth characteristics are not substantially different from those of several other typical members of the genus whose habitats are terrestrial plants or animals, except that in the presence of 5 or 10% sodium chloride on YM agar, growth and pulcherrimin production are particularly abundant, whereas no growth occurs on 50% glucose. Van der Walt and Johannsen (1984) reported variation in the utilization of maltose, trehalose and D-xylose. In this laboratory, the type strain grew weakly on the first two sugars after 3 weeks, but neither strain was seen to assimilate D-xylose.

A moderately close relationship between *K. aestuarii* and certain other *Kluyveromyces* species has long been suspected on the basis of similarity in morphology and physiology (van der Walt 1970c, Martini et al. 1972, Poncet 1973b, Johannsen 1980), including β-galactosidase activity and abundant pulcherrimin formation. *K. aestuarii,* although homothallic, reacts to the *α* mating pheromone of *K. lactis* and forms zygotes with haploid cells of that mating type (Herman 1970). Attempts to document a measurable degree of relatedness by DNA reassociation (Martini 1973, Vaughan-Martini and Martini 1987b, Fuson et al. 1987), immunological cross-reactivity of β-glucanase (Lachance and Phaff 1979), or prototrophic hybrid selection (Johannsen 1980) had negative results, but some recent studies provided such evidence. *K. aestuarii* possesses, in its ribosomal DNA, a *Bam*HI restriction site shared only with *K. lactis, K. marxianus,* and *K. wickerhamii,* and a *Kpn*I site shared only with *K. lactis, K. marxianus, K. dobzhanskii, K. delphensis,* and the multispored *Kluyveromyces* species (Lachance 1989). Also, electrokaryograms of *K. aestuarii* are quite typical

of those of various *K. marxianus* or *K. lactis* strains (Kock et al. 1988, Sor and Fukuhara 1989).

Lactose assimilation combined with lack of growth in the presence of 100 mg/l cycloheximide separate *K. aestuarii* from the rest of the genus.

36.2. *Kluyveromyces africanus* van der Walt (1956b)

Growth on YM agar: After 3 days at 25°C, the cells are ovoidal, (2–5)×(3–8) µm, single, in pairs or in short chains. Conjugating cells may be present. Growth is butyrous, glossy, white to cream-colored.

Growth in glucose–yeast extract broth: A sediment is formed.

Dalmau plate culture on corn meal agar: After two weeks a few chains of cells may be formed.

Formation of ascospores: Asci arise directly from diploid cells. One to 16 or more oblong or reniform ascospores are formed. The spores are liberated from the ascus soon after formation and tend to agglutinate. Early conjugation may be observed.

Asci are formed on YM or malt extract agar after 2–5 days at 17–25°C, but mature ascospores may be difficult to see.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+/w	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	w/–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	–	Cycloheximide 100 mg/l	–
L-Lysine	–	Amino acid-free	w
Ethylamine·HCl	–	Growth at 37°C	w/–
50% Glucose	–		

Co-Q: Not determined.

Mol% G+C: 38.5, CBS 2517 (BD: Fuson et al. 1987).

Origin of the strains studied: CBS 2517, NRRL Y-2404, NRRL Y-2405, NRRL Y-2406, soil, van der Walt, South Africa.

Type strain: CBS 2517, isolated by van der Walt from soil.

Comments: *K. africanus* is represented by four similar strains recovered from South African soil samples. In addition to sharing many phenotypic features with other *Kluyveromyces* species, *K. africanus* is strikingly similar to *Saccharomyces transvaalensis* and *S. dairenensis* when examined by phenetic analysis. A Co-Q 6 yeast isolated from South African soil, *S. transvaalensis* differs by about 4% G+C from *K. africanus* and *S. dairenensis.* Unlike *S. dairenensis, K. africanus* grows weakly on most culture media. In particular, very weak growth is observed on amino acid-free yeast nitrogen base. Growth is stimulated by methionine, less by cysteine.

In addition to the formation of asci with up to 16 (or more) spores, *K. africanus* is resolved from other *Kluyveromyces* species by absence of growth on sucrose, trehalose, and 2-keto-D-gluconate. Separation from *K. phaffii* and *K. yarrowii* by physiological traits is tenuous. *K. phaffii* grows slowly on D-gluconate or ethanol, *K. yarrowii* utilizes ethanol, and *K. africanus* utilizes neither.

36.3. *Kluyveromyces bacillisporus* Lachance, Phaff & Starmer (1993)

Growth on YM agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2–5)×(4–7) µm, single or in pairs. Growth is butyrous, glabrous, and white.

Growth in glucose–yeast extract broth: A sediment is formed.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed.

Formation of ascospores: Asci arise directly from diploid cells (Fig. 103). Four, occasionally 6 to 8 cylindrical to bacilliform ascospores are formed. The spores are liberated from the ascus 1 week after formation and tend to agglutinate.

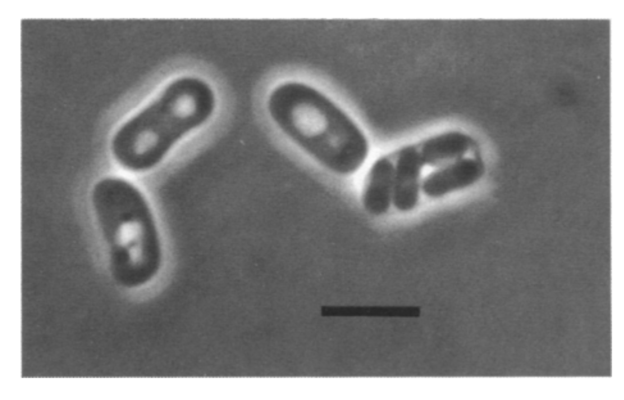


Fig. 103. Asci of *K. bacillisporus*, CBS 7720, after 1 week on McClary's acetate agar. Bar = 5 µm. (Lachance et al. 1993, with permission of the publisher.)

Asci are formed on McClary's acetate agar after 2–5 days at 17–25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	ws	Glycerol	w
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Gelatin liquefaction	–
Cadaverine	–	Cycloheximide 100 mg/l	–
L-Lysine	–	Amino acid-free	+
Ethylamine·HCl	–	Growth at 37°C	w
50% Glucose	–		

Co-Q: Not determined.

Mol% G + C: 37.9–38.1, 2 strains, including CBS 7720 (BD: Lachance et al. 1993).

Origin of the strains studied: CBS 7720 and 2 other strains, exudate, Emory oak (*Quercus emoryi* Torr.), Arizona, U.S.A.

Type strain: CBS 7720, isolated from Emory oak exudate.

Comments: *K. bacillisporus* is represented by three strains recovered from exudates of Emory oak in the vicinity of Tucson, Arizona. These trees were in close proximity to cottonwoods (*Populus fremontii* Wats.) and to various species of prickly pear cacti (*Opuntia* L. spp.), and were being visited by flies (*Drosophila carbonaria* Patterson & Wheeler) that also carried yeasts (Lachance et al. 1993). In view of the fact that these habitats were sampled very extensively, the small number of isolates suggests that the true habitat of *K. bacillisporus* may be another insect or plant. *K. bacillisporus* contains an unusual amount (ca. one half) of mitochondrial DNA. The significance of that observation is not clear.

K. bacillisporus differs from other *Kluyveromyces* species by the utilization of 2-keto-D-gluconate, shared only with *K. blattae,* and the absence of growth on galactose, shared only with *K. delphensis* and *K. waltii.*

36.4. *Kluyveromyces blattae* Henninger & Windisch (1976a)

Growth on YM agar: After 3 days at 25°C, the cells are globose to ellipsoidal, (3–9) × (4–10) μm, single, in pairs or in short chains. Growth is butyrous, glossy, and white to cream-colored.

Growth in glucose–yeast extract broth: A sediment is formed.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed.

Formation of ascospores: Asci arise directly from diploid cells. One to 8 or more spheroidal to ellipsoidal ascospores, occasionally of variable sizes, are formed (Fig. 104). The spores are liberated from the ascus soon after formation and tend to agglutinate.

Immature asci are observed on YM and malt extract agar after 2–5 days at 17–25°C. Ascospores may be seen after 3 weeks.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	w/–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Gelatin liquefaction	–
Cadaverine	–	Cycloheximide 100 mg/l	–
L-Lysine	–	Amino acid-free	–
Ethylamine·HCl	–	Growth at 37°C	–
50% Glucose	–		

Co-Q: Not determined.

Mol% G + C: 34.2, CBS 6284 (BD: Fuson et al. 1987).

Origin of the strains studied: CBS 6284 and CBS 6285, cockroach (*Blatta orientalis* L.), Henninger and Windisch (1976a).

Type strain: CBS 6284, isolated by Henninger and Windisch from a cockroach.

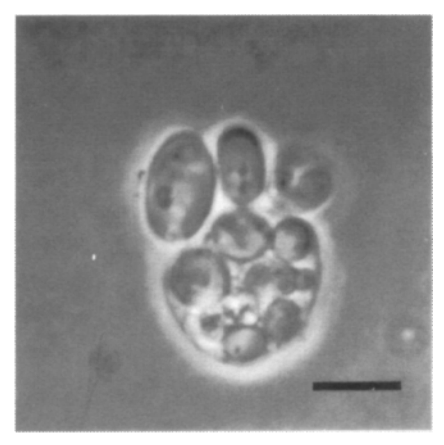


Fig. 104. Ascus of *K. blattae*, CBS 6284, after 3 weeks on YM agar. Bar = 5 μm.

Comments: Henninger and Windisch (1976a) described *K. blattae* based on the isolation of two strains from the oriental cockroach (*Blatta orientalis* L.). An unusual feature of this yeast is the complete failure to grow on minimal medium without amino acids, due to a requirement for histidine. Growth (delayed) has been reported on D-ribose (Barnett et al. 1990), but the observation could not be confirmed in the present study. *K. blattae* shares with *K. bacillisporus* the relatively rare ability to assimilate 2-keto-D-gluconate, but differs from the latter by the absence of galactose assimilation.

36.5. *Kluyveromyces delphensis* (van der Walt & Tseuchschner) van der Walt (1971)

Synonyms:

Saccharomyces delphensis van der Walt & Tscheuschner (1956b)
Dekkeromyces delphensis (van der Walt & Tscheuschner) Novák & Zsolt (1961) nom. inval.
Guilliermondella delphensis (van der Walt & Tscheuschner) Boidin, Abadie, J.L. Jacob & Pignal (1962)
Kluyveromyces delphensis (van der Walt & Tscheuschner) van der Walt (1965c) nom. inval.
Zygofabospora delphensis (van der Walt & Tscheuschner) Naumov (1987b)

Growth on YM agar: After 3 days at 25°C, the cells are ellipsoidal, (1–4) × (2–6) μm, single, in pairs or in short chains. Growth is butyrous, glossy, and cream-colored.

Growth in glucose–yeast extract broth: A sediment and a slight ring are formed.

Dalmau plate culture on corn meal agar: No pseudomycelium is formed.

Formation of ascospores: Asci arise from the conjugation of haploid cells in the same culture. One to four reniform ascospores are formed. The spores are liberated from the ascus soon after formation and tend to agglutinate.

Sporulation occurs after 2–5 days at 17–25°C on YM or malt extract agar.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	s
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	ws	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	w
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	–	Cycloheximide 100 mg/l	–
L-Lysine	ws	Amino acid-free	+
Ethylamine·HCl	–	Growth at 37°C	+
50% Glucose	ws		

Co-Q: Not determined.

Mol% G + C: 40.2, CBS 2170 (BD: Fuson et al. 1987).

Origin of the strain studied: CBS 2170, dried figs, van der Walt and Tscheuschner.

Type strain: CBS 2170, isolated by van der Walt and Tscheuschner from dried figs.

Comments: Van der Walt and Tscheuschner (1956b) isolated *K. delphensis* from dried figs. Due to the availability of only a single strain, it is difficult to comment further on the ecology of this organism. Lachance and Phaff (1979) reported a high degree of similarity between the β-glucanase of that yeast and that of *K. lactis*. Although this has not been investigated further, the possibility of gene flow between different *Kluyveromyces* species is not without precedent (e.g., Johannsen 1980) and should not be discounted. However, genetic exchange probably would not follow the normal path of zygote formation, as evidenced by the negative results obtained by Herman (1970) who showed that mating pheromones of *K. lactis* have no effect on *K. delphensis*.

Phenetically, *K. delphensis* is closest to *K. bacillisporus* and to a lesser extent to other group A species, bearing in addition a superficial resemblance with various nutritionally specialized species of *Saccharomyces*. A previous report of glycerol utilization (van der Walt 1970c) was not confirmed. The combined absence of assimilation of galactose, 2-keto-D-gluconate, and ethylamine sets *K. delphensis* apart from any other *Kluyveromyces* species. Separation from *Saccharomyces* species is based primarily on ascus persistence and ascospore morphology.

36.6. *Kluyveromyces dobzhanskii* (Shehata, Mrak & Phaff) van der Walt (1971)

Synonyms:

Saccharomyces dobzhanskii Shehata, Mrak & Phaff (1955)
Dekkeromyces dobzhanskii (Shehata, Mrak & Phaff) Novák & Zsolt (1961) nom. inval.
Dekkeromyces dobzhanskii (Shehata, Mrak & Phaff) Santa María & Sanchez (1970) nom. inval.
Guilliermondella dobzhanskii (Shehata, Mrak & Phaff) Boidin, Abadie, J.L. Jacob & Pignal (1962)
Kluyveromyces dobzhanskii (van der Walt & Tscheuschner) van der Walt (1965c) nom. inval.
Kluyveromyces marxianus (E.C. Hansen) van der Walt var. *dobzhanskii* (Shehata, Mrak & Phaff) E. Johannsen & van der Walt (Johannsen 1980)
Zygofabospora dobzhanskii (Shehata, Mrak & Phaff) Naumov (1987b)

Growth on YM agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal to cylindrical, (2–6)×(3–11) µm, single, in pairs or in short clusters. Growth is butyrous, glossy, and cream-colored or pink due to pulcherrimin production.

Growth in glucose–yeast extract broth: A sediment and a slight ring are formed.

Dalmau plate culture on corn meal agar: Well ramified pseudomycelium is formed abundantly.

Formation of ascospores: Conjugation of haploid cells in the same culture or bud–parent cell conjugation may precede ascus formation, or asci may arise directly from diploid cells. One to four reniform ascospores are formed. The spores are liberated from the ascus soon after formation and tend to agglutinate.

Sporulation occurs abundantly after 2–5 days at 17–25°C on YM, malt extract, or McClary's acetate agar. Most strains are sporogenous.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	+
Sucrose	+	Trehalose	+
Maltose	+	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	s
D-Xylose	v	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	+	Cycloheximide 100 mg/l	+
L-Lysine	+	Amino acid-free	+
Ethylamine·HCl	+	Growth at 37°C	w/–
50% Glucose	v		

Co-Q: Not determined.

Mol% G+C: 42.6, 2 strains, including CBS 2104 (BD: Fuson et al. 1987).

Origin of the strains studied: CBS 2104, *Drosophila pseudoobscura* Frolova, Shehata et al. (1955); CBS 5061 and CBS 5062, oak white pocket rot mushroom [*Hymenochaeta rubоginosa* (Dickson:Fr.) Lèv.] and brown oak mushroom *Fistulina hepatica* Huds. ex Fr., Boidin, France; climbing shrub [*Actinidia arguta* (Sieb. & Zucc.) Planch. ex Miq.], herbaceous perennial (*Chloranthus japonicus* Sieb.), white nut tree [*Kalopanax septemlobus* (Thunb.) Koidz.], and magnolia vine [*Schisandra chinensis* (Turcz.) Baill.], near Vladivostok, Golubev, USSR; numerous strains from black knot [*Dibotryon morbosum* (Schw.) Theissen & Sydow] of chokecherry (*Prunus virginiana* L.), and from *Drosophila* Fallén spp., Ontario, Canada.

Type strain: CBS 2104, isolated by Shehata et al. (1955) from *Drosophila pseudoobscura.*

Comments: *K. dobzhanskii* is associated with *Drosophila* and related insect habitats such as plants and macrofungi. Very similar in many ways to *K. lactis* var. *drosophilarum,* it nevertheless represents a fully speciated entity, as evidenced by DNA reassociation (Martini 1973, Fuson et al. 1987, Vaughan-Martini and Martini 1987b), by immunological distances of β-glucanases (Lachance and Phaff 1979), and by isoenzyme electrophoresis (Sidenberg and Lachance 1986). *K. dobzhanskii* can be distinguished from other *Kluyveromyces* species by weak growth or absence of growth at 37°C, lack of utilization of lactose, and good growth on β-glucosides (cellobiose and salicin).

36.7. *Kluyveromyces lactis* (Dombrowski) van der Walt (1971)

This species has two varieties:

Kluyveromyces lactis (Dombrowski) van der Walt var. *lactis* (1986)

Anamorph: *Candida spherica* (Hammer & Cordes) S.A. Meyer & Yarrow

Synonyms:

Saccharomyces lactis Dombrowski (1910)
Zygosaccharomyces lactis Dombrowski (1910)
Zygorenospora lactis (Dombrowski) Krasil'nikov (1954a)
Guilliermondella lactis (Dombrowski) Boidin, Abadie, J.L. Jacob & Pignal (1962)
Dekkeromyces lactis (Dombrowski) Santa María & Sanchez (1970) nom. inval.
Kluyveromyces lactis (Dombrowski) van der Walt (1965c) nom. inval.
Kluyveromyces lactis (Dombrowski) van der Walt (1971)
Kluyveromyces marxianus (E.C. Hansen) van der Walt var. *lactis* (Dombrowski) E. Johannsen & van der Walt (Johannsen 1980)

Zygosaccharomyces casei Sacchetti (1932a,b)
Zygosaccharomyces versicolor Sacchetti (1933)
Saccharomyces sociasii C. Ramírez (1954)
Zygofabospora lactis (Dombrowski) Naumov (1987b)
?*Mycoderma lactis* Dombrowski (1910)
Torula sphaerica Hammer & Cordes (1920)
Torulopsis sphaerica (Hammer & Cordes) Lodder (1934)
Cryptococcus sphaericus (Hammer & Cordes) Anderson & Skinner (1947)
Candida spherica (Hammer & Cordes) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Kluyveromyces lactis var. *drosophilarum* (Shehata, Mrak & Phaff) Sidenberg & Lachance (1986)

Synonyms:

Zygosaccharomyces mrakii Shehata & Mrak (1952) nom. inval.
Saccharomyces drosophilarum Shehata, Mrak & Phaff (1955)
Dekkeromyces drosophilarum (Shehata, Mrak & Phaff) Novák & Zsolt (1961) nom. inval.
Guilliermondella drosophilarum (Shehata, Mrak & Phaff) Boidin, Abadie, J.L. Jacob & Pignal (1962)
Dekkeromyces drosophilarum (Shehata, Mrak & Phaff) Santa María & Sanchez (1970) nom. inval.
Kluyveromyces drosophilarum (Shehata, Mrak & Phaff) van der Walt (1965c) nom. inval.
Kluyveromyces drosophilarum (Shehata, Mrak & Phaff) van der Walt (1971)
Saccharomyces drosophilarum (Shehata, Mrak & Phaff) var. *acellobiosus* Phaff, M.W. Miller & Shifrine (1956) nom. inval.
Kluyveromyces marxianus (E.C. Hansen) van der Walt var. *drosophilarum* (Shehata, Mrak & Phaff) E. Johannsen & van der Walt (Johannsen 1980)
Zygofabospora drosophilarum (Shehata, Mrak & Phaff) Naumov (1987b)
Saccharomyces phaseolosporus Shehata, Mrak & Phaff (1955)
Dekkeromyces phaseolosporus (Shehata, Mrak & Phaff) Novák & Zsolt (1961) nom. inval.
Kluyveromyces phaseolosporus (Shehata, Mrak & Phaff) van der Walt (1965c) nom. inval.
Guilliermondella phaseolospora (Shehata, Mrak & Phaff) Boidin, Abadie, J.L. Jacob & Pignal (1962)
Kluyveromyces phaseolosporus (Shehata, Mrak & Phaff) van der Walt (1971)
Zygofabospora phaseolospora (Shehata, Mrak & Phaff) Naumov (1987b)
Zygofabospora krassilnikovii Kudryavtsev (1960)
Dekkeromyces krassilnikovii Kudryavtsev (1960)
Saccharomyces vanudenii van der Walt & Nel (1963)
Kluyveromyces vanudenii (van der Walt & Nel) van der Walt (1965c) nom. inval.
Kluyveromyces vanudenii (van der Walt & Nel) van der Walt (1971)
Kluyveromyces marxianus (E.C. Hansen) van der Walt var. *vanudenii* (van der Walt & Nel) E. Johannsen & van der Walt (Johannsen 1980)

Growth on YM agar: After 3 days at 25°C, the cells are ellipsoidal, (2–6)×(2–8) µm, single, in pairs or in short chains. Growth is butyrous, glossy, cream-colored or pinkish due to pulcherrimin production.

Growth in glucose–yeast extract broth: A sediment and a ring are formed. A thin pellicle is usually formed.

Dalmau plate culture on corn meal agar: A rudimentary pseudomycelium is usually formed.

Formation of ascospores: Conjugation of haploid cells or bud–parent cell conjugation may precede ascus formation, or asci may arise directly from diploid cells. One to four ascospores are formed. The spores range in shape from spheroidal to ellipsoidal to reniform. Sporulating strains of the variety *lactis* are heterothallic, whereas those in the variety *drosophilarum* are homothallic. The spores are liberated from the ascus soon after formation and tend to agglutinate.

Sporulation occurs after 2–5 days at 17–25°C on YM, malt extract, or McClary's acetate agar. Most strains are sporogenous.

Fermentation:

In the fermentation, assimilation and other characteristics, the superscript numbers indicate (1) characteristics that may differ in *K. lactis* var. *drosophilarum*, and (2) characteristics that differ in strain CBS 4693.

Glucose	+	Lactose	$+^1$
Galactose	s	Raffinose	v
Sucrose	v	Trehalose	v
Maltose	v	Inulin	$-^1$

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	$+^1$	Ribitol	v
Trehalose	$+^1$	Galactitol	–
Lactose	$+^1$	D-Mannitol	$+^1$
Melibiose	–	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	$+^1$	Salicin	$+^1$
Inulin	v	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	v	Succinate	+
L-Arabinose	$-^1$	Citrate	$-^1$
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	$+^2$	Cycloheximide 100 mg/l	+
L-Lysine	$+^2$	Amino acid-free	$+^2$
Ethylamine·HCl	$+^2$	Growth at 37°C	$w/-^1$
50% Glucose	v		

Co-Q: 6 (Yamada et al. 1976b).

Mol% G+C: 39.9–40.8, 12 strains, including CBS 683 and CBS 2105 (BD: Fuson et al. 1987).

Supplementary description of *K. lactis* var. *drosophilarum*: The variety *drosophilarum* does not ferment or assimilate lactose. Some strains may ferment inulin or assimilate L-arabinose. Some strains fail to assimilate cellobiose, trehalose, melezitose, mannitol, or salicin. Some strains utilize citrate. All strains grow well at 37°C. The variety *lactis* is heterothallic and the variety *drosophilarum* is homothallic.

Origin of the strains belonging to the variety *lactis*: CBS 683, gassy cheese, Allen and Thornley (1929); CBS

4693, received by CBS from Brust; numerous strains from cheese, Schmidt, France.

Complementary mating types: CBS 683 (α) and CBS 739 (*a*).

Type strain: CBS 683, isolated by Allen and Thornley from gassy cheese.

Origin of the strains belonging to the variety *drosophilarum*: CBS 2105, *Drosophila azteca* Sturtevant & Dobzhansky, Shehata et al. (1955); CBS 4372, type strain of *K. vanudenii,* winery equipment, van der Walt and Nel (1963); CBS 2103, type strain of *K. phaseolosporus, Drosophila pseudoobscura* Frolova, Shehata and Mrak (1952); numerous strains from *Drosophila* spp., black knot [*Dibotryon morbosum* (Schw.) Theissen & Sydow] of chokecherry (*Prunus virginiana* L.), exudate of red oak (*Quercus rubra* L.), Ontario, Canada; *Drosophila carbonaria* Patterson & Wheeler, exudates of Emory oak (*Quercus emoryi* Torr.) and Fremont cottonwood (*Populus fremontii* Wats.), Arizona, Ganter et al. (1986).

Type strain: CBS 2105, isolated by Shehata et al. (1955) from *Drosophila azteca.*

Comments: *K. lactis* exhibits many similarities with several other *Kluyveromyces* species, and is even able, under certain conditions, to form hybrids with them, leading van der Walt and Johannsen (1984) to consider it a variety of *K. marxianus.* The rationale for restoring its full species status is discussed in later sections. On the basis of interfertility and genetic affinities, the former taxa *K. drosophilarum, K. phaseolosporus,* and *K. vanudenii* are considered conspecific with *K. lactis.*

The division of *K. lactis* into the two varieties *lactis* and *drosophilarum* was proposed by Sidenberg and Lachance (1986) and is maintained. It is arbitrarily based on physiological and ecological criteria. Lactose utilizers have arisen almost exclusively in samples from dairy products, whereas lactose-negative strains originate mostly from *Drosophila* or materials known to be *Drosophila* habitats, such as exudates and fungal pathogens of trees. That the same varietal lines seem to be followed by mating compatibility systems may or may not be accidental, but nonetheless adds another basis for maintaining the two infraspecific taxa. A clear genetic partitioning of the species is lacking, and as pointed out by Naumov (1988), division into varieties on the basis of lactose assimilation is not natural. However, until more information is available, a correct genetic definition of varieties within *K. lactis* is not possible. The maintenance of three separate species under the epithets *lactis, drosophilarum,* and *phaseolosporus,* as suggested by Naumov (1988), would not be consistent with the DNA reassociation values ranging from 64 to 100% (Martini 1973, Fuson et al. 1987) among representatives of these taxa and of the former designation *K. vanudenii.* The reassociation values are independent of former taxonomic assignments. For example, two strains previously assigned to *K. vanudenii* gave, respectively, 98% and 68% heteroduplex formation with the type of *K. lactis* var. *lactis* (Fuson et al. 1987). The type strain of *K. phaseolosporus* gave 90% reassociation with the type of the variety *lactis* and 83% with the type of the variety *drosophilarum.* Seven strains formerly designated as *K. drosophilarum* had reassociation values ranging from 64 to 80% with the type of the variety *lactis,* and from 64% to 86% with the type of the variety *drosophilarum.* The type cultures of the two recognized varieties formed 77% and 80% heteroduplexes in reciprocal experiments. Electrokaryotypes (Sor and Fukuhara 1989) are equally perplexing. Although different strains have important differences in chromosome size distributions between the varieties, significant variation exists within the varieties as well.

The conspecificity of *Candida spherica* and *K. lactis,* recognized by van der Walt (1970c), has been confirmed by Vaughan-Martini and Martini (1985) by means of DNA reassociation.

K. lactis is rapidly becoming a serious competitor to *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* as a model organism. A European-based, international group of geneticists interested in *K. lactis* has met in 1990 and agreed to adopt a standard nomenclature of mutations and chromosomes for the species (H. Fukuhara and B.J.M. Zonneveld, personal communications). They have chosen strain CBS 2359 as standard for genetic analysis. As an example of the work being done with *K. lactis*, a circular plasmid of the so-called 2μ family has been isolated from *K. lactis* var. *drosophilarum* (Falcone et al. 1986), and used to create a gene cloning system for *K. lactis* (Chen et al. 1988). Interestingly, the plasmid could be replicated in both varieties of *K. lactis,* but not in *K. marxianus.* Linear killer plasmids of *K. lactis* have also been used to construct vectors (Kamper et al. 1989).

K. lactis has also been explored for its industrial potential. For example, the β-galactosidase from *K. lactis* can be used to reduce the lactose content of milk (Hussein et al. 1989).

For identification purposes, *K. lactis* var. *lactis* is unique in the genus in assimilating both lactose and α-glucosides. *K. lactis* var. *drosophilarum* is best recognized, among lactose-negative species that utilize ethylamine and L-lysine strongly, by its vigorous growth at 37°C.

36.8. *Kluyveromyces lodderae* (van der Walt & Tscheuschner) van der Walt (1971)

Synonyms:

Saccharomyces lodderae (as *S. lodderi*) van der Walt & Tscheuschner (1957a)

Dekkeromyces lodderi (van der Walt & Tscheuschner) Novák & Zsolt (1961) nom. inval.

Guilliermondella lodderi (van der Walt & Tscheuschner) Boidin, Abadie, J.L. Jacob & Pignal (1962)

Kluyveromyces lodderi (van der Walt & Tscheuschner) van der Walt (1965c) nom. inval.

Zygofabospora lodderi (van der Walt & Tscheuschner) Naumov (1987b)

Growth on YM agar: After 3 days at 25°C, the cells

are ellipsoidal to cylindrical, (2–6)×(3–11) μm, single, in pairs or in small clusters. Growth is butyrous, glossy to dull, and cream-colored.

Growth in glucose–yeast extract broth: A sediment and a slight ring are formed.

Dalmau plate culture on corn meal agar: A rudimentary pseudomycelium is formed.

Formation of ascospores: Conjugation of haploid cells or bud–parent cell conjugation may precede ascus formation, or asci may arise directly from diploid cells. One to four reniform or bacilliform ascospores are formed. The spores are liberated from the ascus soon after formation and tend to agglutinate.

Sporulation occurs after 1 week at 17–25°C on 1% malt extract agar and McClary's acetate agar. Strain UCD-FST 70-3, a subculture of the type, sporulates better than other cultures examined.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	+
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	s
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	w/–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	s	Cycloheximide 100 mg/l	+
L-Lysine	–	Amino acid-free	+
Ethylamine·HCl	–[1]	Growth at 37°C	–
50% Glucose	–		

[1] A slow, but positive reaction was observed in the type culture.

Co-Q: Not determined.

Mol% G+C: 35.4–35.7, 2 strains, including CBS 2757 (BD: Fuson et al. 1987).

Origin of the strains studied: CBS 2757 [UCD-FST 70-3], soil, van der Walt and Tscheuschner, NRRL YB-4303, NRRL YB-4304, NRRL YB-4305, NRRL YB-4306, soil, van der Walt, South Africa.

Type strain: CBS 2757, isolated by van der Walt and Tscheuschner from soil.

Comments: This species was originally described as *K. lodderi,* but, as pointed out by Barnett et al. (1983), and in accordance to Article 73 of the Botanical Code (Greuter et al. 1988), the correct Latin orthography of the epithet *lodderae* should be used. *K. lodderae* was recovered from soil, but its true habitat is not known. *K. lodderae* is phenetically similar in many ways with *K. polysporus,* but the two are easily distinguished on the basis of ascospore numbers. In addition, unlike *K. polysporus, K. lodderae* assimilates cadaverine vigorously, albeit slowly. Strain NRRL YB-4306 gave weaker reactions than the type for the assimilation of glycerol, lactic and succinic acids, and growth in the absence of amino acids. The slow assimilation of inulin reported by van der Walt (1970c) could not be confirmed. The utilization of ethylamine as sole nitrogen source, reported as positive by Barnett et al. 1990, was only observed in the type culture, and growth on lysine, reported as variable by these authors, was negative.

K. lodderae differs from species of *Kluyveromyces* that fail to grow on both ethylamine and L-lysine by the utilization of the β-fructosides sucrose and raffinose, and the ability to grow in the presence of 100 mg/l cycloheximide.

36.9. *Kluyveromyces marxianus* (E.C. Hansen) van der Walt (1971)

Anamorph: *Candida kefyr* (Beijerinck) van Uden & H.R. Buckley

Synonyms:

Saccharomyces marxianus E.C. Hansen (1888b)
Zygosaccharomyces marxianus (E.C. Hansen) Guilliermond & Negroni (1929)
Zygorenospora marxiana (E.C. Hansen) Krasil'nikov (1954a)
Fabospora marxiana (E.C. Hansen) Kudryavtsev (1960) nom. inval.
Dekkeromyces marxianus (E.C. Hansen) Novák & Zsolt (1961) nom. inval.
Guilliermondella marxiana (E.C. Hansen) Boidin, Abadie, J.L. Jacob & Pignal (1962)
Kluyveromyces marxianus (E.C. Hansen) van der Walt (1965c) nom. inval.
Kluyveromyces marxianus (E.C. Hansen) var. *marxianus* (E.C. Hansen) E. Johannsen & van der Walt (Johannsen 1980)
Zygofabospora marxiana (E.C. Hansen) Naumov (1987b)
Saccharomyces cavernicola Redaelli (1925)
Saccharomyces fragilis (Jörgensen) var. *bulgaricus* Santa María (1956b)
Kluyveromyces cicerisporus van der Walt, Nel & van Kerken (1966)
Kluyveromyces bulgaricus (Santa Maria) van der Walt (1970c) nom. inval.
Kluyveromyces bulgaricus (Santa María) van der Walt (1971)
Kluyveromyces marxianus (E.C. Hansen) van der Walt var. *bulgaricus* (Santa María) E. Johannsen & van der Walt (Johannsen 1980)
Kluyveromyces wikenii van der Walt, Nel & van Kerken (1966)
Kluyveromyces marxianus (E.C. Hansen) van der Walt var. *wikenii* (van der Walt, Nel & van Kerken) E. Johannsen & van der Walt (Johannsen 1980)
Saccharomyces fragrans Beijerinck (1895)
Saccharomyces muciparus Beijerinck (1908)
Saccharomyces fragilis Jörgensen (1909)
Zygorenospora fragilis (Jörgensen) Krasil'nikov (1954a)
Fabospora fragilis (Jörgensen) Kudryavtsev (1960) nom. inval.
Dekkeromyces fragilis (Jörgensen) Novák & Zsolt (1961) nom. inval.
Guilliermondella fragilis (Jörgensen) Boidin, Abadie, J.L. Jacob & Pignal (1962)

Dekkeromyces fragilis (Jörgensen) Santa María & Sanchez (1970) nom. inval.
Kluyveromyces fragilis (Jörgensen) van der Walt (1965c) nom. inval.
Kluyveromyces fragilis (Jörgensen) van der Walt (1971)
Zygosaccharomyces ashbyi Cordro'ch (1937) nom. inval.
Saccharomyces macedoniensis Diddens & Lodder (1939)
Fabospora macedoniensis (Diddens & Lodder) Kudryavtsev (1960) nom. inval.
Dekkeromyces macedoniensis (Diddens & Lodder) Novák & Zsolt (1961) nom. inval.
Saccharomyces chevalieri (Guilliermond) var. *atypicus* Dietrichson (1954) nom. inval.
Hansenula pozolis Herrera, Ulloa & Fuentes (1973)
Saccharomyces kefyr Beijerinck (1889b)
Mycotorula kefyr (Beijerinck) Harrison (1928)
Geotrichoides kefyr (Beijerinck) Langeron & Talice (1932)
Torulopsis kefyr (Beijerinck) Lodder (1934)
Cryptococcus kefyr (Beijerinck) Skinner (1947a)
Candida kefyr (Beijerinck) van Uden & H.R. Buckley (1970)
Mycotorula lactis Trüper (1928)
Mycotorula lactosa Harrison (1928)
Candida pseudotropicalis (Castellani) Basgal var. *lactosa* (Harrison) Diddens & Lodder (1942)
Endomyces pseudotropicalis Castellani (1911)
Monilia pseudotropicalis (Castellani) Castellani & Chalmers (1913)
Atelosaccharomyces pseudotropicalis (Castellani) Froilano de Mello & Gonzaga Fernandes (1918)
Myceloblastanon pseudotropicalis (Castellani) Ota (1928)
Mycocandida pseudotropicalis (Castellani) Ciferri & Redaelli (1935)
Castellania pseudotropicalis (Castellani) Dodge (1935)
Monilia pseudotropicalis (Castellani) Castellani & Chalmers (Castellani 1937a)
Monilia pseudotropicalis (Castellani) Castellani & Chalmers var. *metapseudotropicalis* Castellani (1937a)
Mycotorula pseudotropicalis (Castellani) Redaelli & Ciferri (1947)
Candida pseudotropicalis (Castellani) Basgal (1931)
Cryptococcus sulphureus Beauverie & Lesieur (1912)
Monilia sulphurea (Beauverie & Lesieur) Vuillemin (1931)
Torula cremoris Hammer & Cordes (1920)
Candida mortifera Redaelli (1925)
Mycocandida mortifera (Redaelli) Langeron & Talice (1932)
Monilia mortifera (Redaelli) Martin, Jones, Yao & Lee (1937)
Candida mortifera (Redaelli) var. α Redaelli (1925)
Monilia macedoniensis Castellani & Chalmers (1919)
Candida macedoniensis (Castellani & Chalmers) Berkhout (1923)
Myceloblastanon macedoniensis (Castellani & Chalmers) Ota (1928)
Blastodendrion macedoniensis (Castellani & Chalmers) Langeron & Guerra (1935)
Mycotorula macedoniensis (Castellani & Chalmers) Redaelli & Ciferri (Ciferri and Redaelli 1935)
Castellania macedoniensis (Castellani & Chalmers) Dodge (1935)
Mycotoruloides macedoniensis (Castellani & Chalmers) Dodge & Moore (1936)
Monilia macedoniensoides Castellani & Taylor (1925)
Castellania macedoniensoides (Castellani & Taylor) Dodge (1935)
Cryptococcus kartulisi Castellani (1928)
Castellania kartulisi (Castellani) Dodge (1935)
Blastodendrion procerum Zach (Wolfram and Zach 1934b)
Mycocandida pinoysimilis (Castellani) Redaelli & Ciferri var. *citelliana* Redaelli & Ciferri (Ciferri and Redaelli 1935)
Pseudomycoderma mazzae Dodge (1935)

Growth on YM agar: After 3 days at 25°C, the cells are globose, ellipsoidal, to cylindrical, $(2–6)\times(3–11)\,\mu m$, single, in pairs or in short chains. Growth is butyrous, glossy, cream-colored to brown, rarely pinkish due to pulcherrimin production.

Growth in glucose–yeast extract broth: A sediment and a ring are formed. A thin pellicle may be formed.

Dalmau plate culture on corn meal agar: A rudimentary to highly branched pseudomycelium with a few blastospores may be formed.

Formation of ascospores: Conjugation of haploid cells or bud–parent cell conjugation may precede ascus formation, or asci may arise directly from diploid cells. One to four ascospores are formed. The spores range in shape from spheroidal to ellipsoidal to reniform. Sporulating cultures appear to be homothallic. The spores are liberated from the ascus soon after formation and tend to agglutinate.

Sporulation occurs after 2–5 days at 17–25°C on 1% malt extract agar and McClary's acetate agar. Most strains sporulate well.

Fermentation:

Glucose	+	Lactose	v
Galactose	s	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–	Inulin	s

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	s	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	v	Ribitol	s
Trehalose	w/–	Galactitol	–
Lactose	v	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	+	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	s	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	+	Cycloheximide 100 mg/l	+
L-Lysine	+	Amino acid-free	+
Ethylamine·HCl	+	Growth at 37°C	+
50% Glucose	–		

Co-Q: 6 (Yamada et al. 1976b).

Mol% G+C: 41.1–41.5, 12 strains, including CBS 712 (BD: Fuson et al. 1987).

Origin of the strains studied: CBS 712, Schnegg; CBS 2762, type strain of *K. bulgaricus,* yogurt, Santa María; CBS 4857, type strain of *K. cicerisporus*; CBS 397, type strain of *K. fragilis,* yogurt; CBS 5671, type strain of *K. wikenii,* sorghum beer, van der Walt et al. (1966); numerous strains from cheese, Schmidt, France; one strain from prickly pear (*Opuntia phaeacantha* Engelmann), and one from *Drosophila carbonaria* Patterson & Wheeler, Arizona, Ganter et al. (1986).

Type strain: CBS 712 (neotype), Schnegg.

Comments: In the last monograph on *Kluyveromyces* (van der Walt and Johannsen 1984), the definition of *K. marxianus* was altered to reflect the ability of certain species to hybridize. Following that view, *K. marxianus* encompassed several varieties, among which were taxa recognized here as either distinct species *(K. dobzhanskii, K. lactis),* or synonyms of *K. lactis* or *K. marxianus*. The species definition used in the present treatment takes into account hybridization ability in combination with the genetic structure of populations. Because species delineation in *K. marxianus* has been controversial, these criteria are now given explicitly.

The biological species concept is unequivocally applicable to yeasts only when they are heterothallic. The majority of *Kluyveromyces* species are homothallic and thus facultatively self-fertile. For such yeasts, whether or not populations effectively share a common gene pool depends not only on mating potential, but also on numerous other factors that are difficult to ascertain. However, the end result of evolution can be examined to see whether genes are patterned consistently with a history of phyletic divergence, or whether they exhibit a diffuse genetic clustering indicative of panmixia (random mating). The available information on physiological properties, isoenzyme distributions, ecological affinities, and nuclear genome DNA heteroduplex formation (see Comments on the genus) clearly indicates that *K. marxianus* is evolutionarily indistinct from taxa once designated by the epithets *bulgaricus, cicerisporus, fragilis,* or *wikenii,* and that it has speciated fully from *K. dobzhanskii* and *K. lactis*. The distinct nature of *K. marxianus* and *K. lactis* has received further substantiation most recently from the work of Steensma et al. (1988), who examined, by electrokaryotyping and phenotype analysis, hybrids of the two species and single spore isolates obtained from the hybrids. The spores had low viability, but more importantly, the few viable spores grew into cultures that were identical, in karyotype or genotype, to either the hybrid or one of the parents. Balanced genetic recombination was not taking place.

If *K. lactis* is rapidly stealing from *Saccharomyces cerevisiae* its place in the limelight of molecular genetics, *K. marxianus* is competing for the status of most celebrated domestic microbe. The examples given below should be viewed as a minute sampling of a vast literature. The lactose hydrolysing properties of *K. marxianus* (and *K. lactis*) have long been known for their potential in the food industry. Interestingly, Algeri et al. (1978) observed that lactose uptake and β-galactosidase activity in *K. marxianus* are induced by lactose whereas they are constitutive in *K. lactis,* based on an examination of one strain of each species. Carvalho-Silva and Spencer-Martins (1990) compared several strains of *K. marxianus* and *K. lactis* on the basis of several features of their lactose uptake, and found that they could be assigned to three different groups that were unfortunately not congruent with their species assignments.

Many publications in the last ten years describe studies of inulin utilization by *K. marxianus.* Interest in this area stems from the abundance of inulin in certain easily grown crop plants such as the Jerusalem artichoke *(Helianthus tuberosus).* At the risk of being unjust to all others, a single recent study will be cited in which several key reports are reviewed. Strain CBS 6556, originally described as *Hansenula pozolis* by Herrera et al. (1973), was found to combine thermotolerance with higher productivity when compared with other inulinase-producing strains of *K. lactis* (including representatives of former taxon *K. vanudenii*) and *K. marxianus* (Rouwenhorst et al. 1988). Inulinase, in that yeast, is bound to the cell envelope, but can be released at higher pH or under reducing conditions. Its synthesis is repressed by glucose or lactose, and reaches a maximum at high temperatures (37–42°C).

The extracellular pectinolytic activity of *K. marxianus* endows it with the potential to be both useful and detrimental to human activity. In the first instance, the endo-polygacturonase of this yeast has been used experimentally to eliminate the pectin haze of apple juice (Gomez-Ruiz et al. 1988). *K. marxianus* has also been identified as the agent of a soft rot in onion (Johnson et al. 1988). *K. marxianus* is able to produce glycerol from lactose when grown in whey permeate if the medium is supplemented with 1% Na_2SO_3 (Jeng et al. 1989).

The thermotolerance of *K. marxianus* allows it to be used in very rapid processes of ethanol production, which might compensate for its lower tolerance to ethanol compared to *S. cerevisiae*. Anderson et al. (1986) obtained 6% ethanol at 43°C in one day with isolates selected from sugar mill effluents. Interestingly, the better isolates keyed to *K. marxianus* var. *marxianus* (*sensu* van der Walt & Johannsen 1984). The type culture of the former *K. bulgaricus* gave less satisfactory results, and those of *K. lactis* and *K. wickerhamii* gave poor results.

Bak and Stenderup (1969) demonstrated the existence of a high degree of genetic relatedness between *K. fragilis* (syn. *K. marxianus*) and *Candida pseudotropicalis,* now considered a synonym of *Candida kefyr.* Vaughan-Martini and Martini (1985) confirmed the very high genetic relatedness of these taxa.

Yeasts with significant biotechnological interest are particularly difficult to characterize ecologically. The β-galactosidase activity of *K. marxianus* is likely to explain its frequent recovery from samples of materials associated with warm-blooded animals, including dairy products. A number of isolates, some of which do not utilize lactose, originate from natural fermentations.

The fact that *K. marxianus* was once represented by five different nomenclatural species reflects the phenetic heterogeneity of the species, and explains why it is difficult to distinguish from other related taxa, especially *K. lactis*. However, *K. marxianus* is the only inulin-assimilating *Kluyveromyces* species that does not assimilate or ferment α-glucoside and grows well at 37°C.

36.10. *Kluyveromyces phaffii* (van der Walt) van der Walt (1971)

Synonyms:

Fabospora phaffii van der Walt (1963)

Kluyveromyces phaffii (van der Walt & Tscheuschner) van der Walt (1965c) nom. inval.

Growth on YM agar: After 3 days at 25°C, the cells are ellipsoidal to cylindrical, (2–4)×(3–8) µm, single, in pairs or in small clusters. Growth is butyrous, glossy to dull, and cream-colored.

Growth in glucose–yeast extract broth: A sediment is formed.

Dalmau plate culture on corn meal agar: Short chains of cells may be formed.

Formation of ascospores: Asci arise directly from diploid cells. One to four reniform or bacilliform ascospores are formed. The spores are liberated from the ascus soon after formation and tend to agglutinate.

Sporulation occurs after 2–5 days at 17–25°C on YM, malt extract, or McClary's acetate agar.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	–	Cycloheximide 100 mg/l	–
L-Lysine	–	Amino acid-free	+
Ethylamine·HCl	–	Growth at 37°C	–
50% Glucose	–		

Co-Q: Not determined.

Mol% G+C: 35.3, CBS 4417 (BD: Fuson et al. 1987).

Origin of the strain studied: CBS 4417, soil, van der Walt.

Type strain: CBS 4417, isolated by van der Walt from soil.

Comments: *K. phaffii* differs from *K. africanus* and *K. blattae* mostly by ascospore numbers and from *K. yarrowii* and others largely by ploidy of the natural isolates. These two characters were considered sufficient by Naumov (1987b) to propose the reclassification of *K. phaffii* in a separate genus. However, taking into account all the phenetic and genetic data available, it is clear that the current classification is satisfactory.

Physiologically, lack of ethylamine utilization, absence of growth on β-fructosides, and assimilation of D-gluconate, set *K. phaffii* apart from other galactose-utilizing *Kluyveromyces* species.

36.11. *Kluyveromyces polysporus* van der Walt (1956a)

Synonym:

Saccharomyces polysporus (van der Walt) Campbell (1972)

Growth on YM agar: After 3 days at 25°C, the cells are globose to cylindrical, (2–5)×(3–7) µm, single, in pairs or in short chains. Conjugating cells and large zygotes may be present. Growth is butyrous, glossy, cream-colored to brownish.

Growth in glucose–yeast extract broth: A sediment and a thin pellicle are formed.

Dalmau plate culture on corn meal agar: Chains of cells or rudimentary pseudomycelium may be formed.

Formation of ascospores: Asci arise from the conjugation of haploid cells. Large numbers of oblong to reniform ascospores are formed (Fig. 105). The spores are liberated from the ascus soon after formation and tend to agglutinate.

The type culture forms abundant asci after 1 week at 17–25°C on YM agar. It is difficult to observe mature asci because the ascospores are released easily.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	s
Maltose	–	Inulin	–

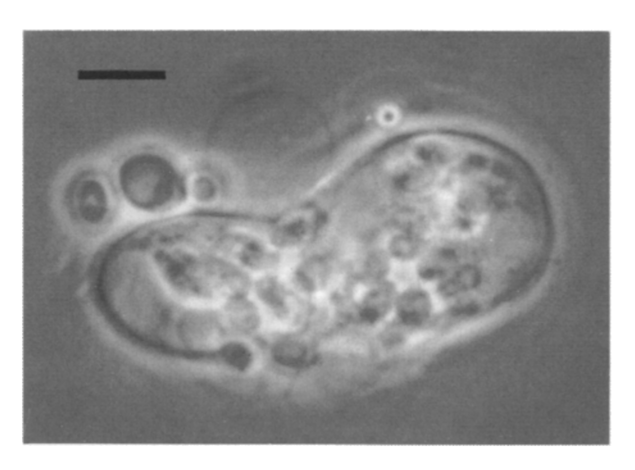


Fig. 105. Lysed ascus of *K. polysporus*, CBS 2163, after 1 week on YM agar. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w/–
D-Xylose	–	Succinate	ws
L-Arabinose	–	Citrate	s
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	–	Cycloheximide 100 mg/l	–
L-Lysine	–	Amino acid-free	+
Ethylamine·HCl	–	Growth at 37°C	w/–
50% Glucose	–		

Co-Q: Not determined.
Mol% G+C: 35.3, CBS 2163 (BD: Fuson et al. 1987).
Origin of the strains studied: CBS 2163, soil, South Africa; NRRL Y-2397, NRRL Y-2400, van der Walt.

Type strain: CBS 2163, isolated by van der Walt from soil.

Comments: The type species of *Kluyveromyces, K. polysporus* is unique among yeasts because of its ability to produce up to 100 ascospores due to supernumerary mitoses in the ascus parent cell (Roberts and van der Walt 1958). Van der Walt (1956a) hypothesized that the multispored state was an evolutionary carry-over from more developed fungi into the realm of yeasts, but that the vigorously fermentative uninucleate nature of *K. polysporus* made it a bona fide member of the Endomycetales. The later realization that other yeast species are phenetically close to *K. polysporus* confirmed the correctness of the assignment.

Physiologically, *K. polysporus* exhibits a particularly high degree of similarity to *K. lodderae,* but differs from the latter by growth on citric and D-gluconic acids, and failure to grow in the presence of cycloheximide (100 mg/l). *K. polysporus* differs from all other ethylamine-negative *Kluyveromyces* species by the utilization of β-fructosides and absence of growth on 2-keto-D-gluconate.

36.12. *Kluyveromyces thermotolerans* (Filippov) Yarrow (1972)

Synonyms:
Zygosaccharomyces thermotolerans Filippov (1932)
Saccharomyces thermotolerans (Filippov) Campbell (1972)
Zygofabospora thermotolerans (Filippov) Naumov (1987b)
Torula dattila Kluyver (1914) (see van der Walt and E. Johannsen 1974)
Mycotorula dattila (Kluyver) Harrison (1928)
Torulopsis dattila (Kluyver) Lodder (1934)
Cryptococcus dattilus (Kluyver) Skinner (1947a)
Candida dattila (Kluyver) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Saccharomyces veronae Lodder & Kreger-van Rij (1952)
Kluyveromyces veronae (Lodder & Kreger-van Rij) van der Walt (1970c) nom. inval.
Kluyveromyces veronae (Lodder & Kreger-van Rij) van der Walt (1971)
Zygosaccharomyces drosophilae Shehata & Mrak (1952) nom. inval.

Growth on YM agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (3–6)×(6–8) µm, single, in pairs or in short clusters. Growth is butyrous, glossy, and cream-colored.
Growth in glucose–yeast extract broth: A sediment and a slight ring are formed. Occasionally, growth may be very flocculent.
Dalmau plate culture on corn meal agar: Pseudomycelium may be formed. When present, it ranges from very rudimentary to highly branched with blastospores.
Formation of ascospores: Conjugation of haploid cells in the same culture or bud–parent cell conjugation may precede ascus formation, or asci may arise directly from diploid cells. One to four spherical ascospores with conspicuous lipid globules are formed. The spores are liberated from the ascus soon after formation and tend to agglutinate.

Sporulation occurs abundantly in most strains after 2–5 days at 17–25°C on YM, malt extract, or McClary's acetate agar.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	+
Sucrose	+	Trehalose	s
Maltose	v	Inulin	v

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	s
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	v	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	v	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Gelatin liquefaction	–
Cadaverine	+	Cycloheximide 100 mg/l	–
L-Lysine	+	Amino acid-free	+
Ethylamine·HCl	+	Growth at 37°C	v
50% Glucose	+		

Co-Q: 6 (Yamada et al. 1976b).
Mol% G+C: 46.2, CBS 2803 (BD: Fuson et al. 1987).

Origin of the strains studied: CBS 6340, plum jam, Filippov (1932); CBS 2803, type of *Kluyveromyces veronae* (van der Walt 1971), grapes, Verona; CBS 137, type of *Candida dattila;* many strains from black knot [*Dibotryon morbosum* (Schw.) Theissen & Sydow] of sand cherry (*Prunus pumila* L.) and chokecherry (*Prunus virginiana* L.), *Drosophila* Fallén spp., and insect galls of red oak (*Quercus rubra* L.), Ontario, Canada; black knot of black cherry (*Prunus serotina* Ehrh.), Québec, Canada; fruit of prickly pear (*Opuntia* L. sp.), Big Pine Key, Florida; fruit of a cereoid cactus, Shroud Cay, Exuma Cays, Bahamas; a drosophilid (*Gitona americana* Patterson) collected near decaying prickly pear (*Opuntia stricta* Haworth) somatic tissue, Cayman Islands; *Drosophila carbonaria* Patterson & Wheeler, Arizona (Ganter et al. 1986).

Type strain: CBS 6340, isolated by Filippov from fermenting plum jam.

Comments: *Saccharomyces veronae* was transferred to *Kluyveromyces* "provisionally and for purely practical reasons" by van der Walt (1970c). When Yarrow (1972) later studied authentic strains of *Zygosaccharomyces thermotolerans* and found them to be indistinguishable from *K. veronae,* he assigned the fused species to *Kluyveromyces* because he observed ascus deliquescence. This does not alter the fact that *K. thermotolerans* has obvious natural affinities with *Saccharomyces* and *Zygosaccharomyces,* a property that is shared with *K. waltii.* They are retained in *Kluyveromyces* monothetically on the basis of ascus deliquescence. Ascospore ultrastructure in *K. thermotolerans* and *K. waltii* is similar to that of certain *Zygosaccharomyces* species and different from that of *K. marxianus,* but equally important differences are observed within the genus *Zygosaccharomyces* itself (Kreger-van Rij 1979). Phenetically, *K. thermotolerans* is very similar not only to *Zygosaccharomyces florentinus* and *Zygosaccharomyces cidri,* but also to *Saccharomyces kluyveri.* However, an equally high degree of similarity exists between *Zygosaccharomyces fermentati* and *K. dobzhanskii,* and to a lesser extent between *Z. fermentati* and *K. lactis* or *K. marxianus.* Although it is possible that *K. thermotolerans* may one day return to one of its genera of origin, carrying along with it *K. waltii,* the choice of which genus is not at all obvious. The arguments in favor of transferring them at this time are not at all compelling.

Candida dattila was found by van der Walt and Johannsen (1974) to produce ascospores after a suitable heat treatment. They noted the very high phenetic similarity between *C. dattila* and *K. thermotolerans,* and concluded that the two taxa were synonyms. Vaughan-Martini and Martini (1985) demonstrated a high degree of DNA reassociation between these taxa.

K. thermotolerans is associated with fruit, *Drosophila* species, and possibly other insects that use plants for feeding purposes. Two unusual strains were recovered from drosophilid flies collected near decaying prickly pear tissue in the Cayman Islands. Both strains utilized melibiose, and one strain grew vigorously at 37°C. The utilization of 2-keto-D-gluconate by some strains (including the type) and not by others is unusual, as that compound generally appears to be taxonomically stable.

For identification purposes, *K. thermotolerans* can be recognized among species that utilize ethylamine and L-lysine, by its combined lack of growth on β-glucosides or in the presence of 100 mg/l cycloheximide.

36.13. *Kluyveromyces waltii* K. Kodama (1974)

Synonym:

Zygofabospora waltii (K. Kodama) Naumov (1986a)

Growth on YM agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (3–6)×(4–10) μm, single, in pairs or in short clusters. Growth is butyrous, glossy, and cream-colored.

Growth in glucose–yeast extract broth: A sediment is formed.

Dalmau plate culture on corn meal agar: Pseudomycelium may be formed. When present, it ranges from very rudimentary to highly branched with blastospores.

Formation of ascospores: Conjugation of haploid cells in the same culture or bud–parent cell conjugation precedes ascus formation. One to four spherical ascospores with lipid globules are formed. The spores are liberated slowly from the ascus and tend to agglutinate.

Sporulation occurs after 2–5 days at 17–25°C on YM, malt extract, or McClary's acetate agar. All strains sporulate moderately well.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	s
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	s	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	+	Cycloheximide 100 mg/l	+
L-Lysine	+	Amino acid-free	+
Ethylamine·HCl	+	Growth at 37°C	w/–
50% Glucose	ws		

Co-Q: Not determined.
Mol% G+C: 45.7, CBS 6430 (BD: Fuson et al. 1987).

Origin of the strains studied: CBS 6430 [UCD-FST 72-13], exudate of holly (*Ilex* L. sp.), Kodama, Japan; several strains were recovered from *Drosophila* Fallén spp., black knot [*Dibotryon morbosum* (Schw.) Theissen & Sydow] of chokecherry (*Prunus virginiana* L.), and insect galls of red oak (*Quercus rubra* L.), Ontario, Canada.

Type strain: CBS 6430, isolated by Kodama from holly exudate.

Comments: As noted for *K. thermotolerans*, the inclusion of *K. waltii* in *Kluyveromyces* is not based on solid evidence of monophyly. *K. waltii* exhibits greater phenetic resemblance to *Zygosaccharomyces mrakii* and *Zygosaccharomyces microellipsoides* than to any *Kluyveromyces* species. Deliquescence of its asci requires considerably more time compared to other species, including *K. thermotolerans*. Notwithstanding these considerations, the current classification is retained, because revisions should hinge on compelling evidence which is not currently available.

K. waltii is probably insect associated, having been recovered from plant injuries known or suspected to serve as feeding sites for *Drosophila* species and other insects. *K. waltii* differs from other ethylamine- and L-lysine-assimilating species of *Kluyveromyces* by the inability to assimilate galactose and the ability to grow in the presence of 100 mg/l cycloheximide.

36.14. *Kluyveromyces wickerhamii* (Phaff, M.W. Miller & Shifrine) van der Walt (1971)

Synonyms:
Saccharomyces wickerhamii Phaff, M.W. Miller & Shifrine (1956)
Dekkeromyces wickerhamii (Phaff, M.W. Miller & Shifrine) Novák & Zsolt (1961) nom. inval.
Guilliermondella wickerhamii (Phaff, M.W. Miller & Shifrine) Boidin, Abadie, J.L. Jacob & Pignal (1962)
Dekkeromyces wickerhamii (Phaff, M.W. Miller & Shifrine) Santa María & Sanchez (1970) nom. inval.
Kluyveromyces wickerhamii (Phaff, M.W. Miller & Shifrine) van der Walt (1965c) nom. inval.
Zygofabospora wickerhamii (Phaff, M.W. Miller & Shifrine) Naumov (1986a)

Growth on YM agar: After 3 days at 25°C, the cells are spheroidal to cylindrical, (2–4)×(3–6) μm, single, in pairs or in short clusters. Growth is butyrous, glossy, and cream-colored or pink to brown because of abundant pulcherrimin production.

Growth in glucose–yeast extract broth: A sediment and a thin, dull pellicle are formed.

Dalmau plate culture on corn meal agar: A rudimentary pseudomycelium is formed.

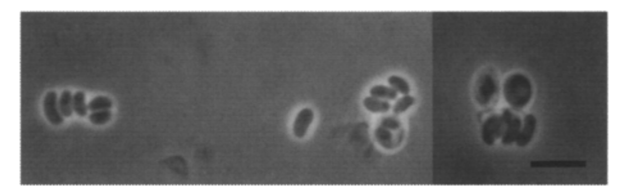


Fig. 106. Asci and free ascospores of *K. wickerhamii*, UWO(PS)91-127.2, after 2 weeks on YM agar. Bar = 5 μm.

Formation of ascospores: Conjugation of haploid cells in the same culture may precede ascus formation, or asci may arise directly from diploid cells. One to four reniform to crescentiform ascospores are formed (Fig. 106). The spores are liberated from the ascus soon after formation and tend to agglutinate.

Sporulation is abundant for most strains after 2–5 days at 17–25°C on YM, malt extract, or McClary's acetate agar.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	s	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+/w
Sucrose	+	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	+/w	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	+	D-Mannitol	–
Melibiose	–	D-Glucitol	ws
Raffinose	v	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	+	Cycloheximide 100 mg/l	+
L-Lysine	+	Amino acid-free	+
Ethylamine·HCl	+	Growth at 37°C	v
50% Glucose	–		

Co-Q: Not determined.
Mol% G+C: 41.9–42.4, 5 strains, including CBS 2745 (BD: Fuson et al. 1987).

Origin of the strains studied: CBS 2745 [NRRL Y-8286, UCD-FST 54-210], *Drosophila pinicola* Sturtevant, Sierra Nevada Mountains, California, Phaff, USA; NRRL Y-5973 [UCD-FST 56-40], slime flux of fir tree (*Abies* L. sp.), Miller, USA; UWO(PS)85-330.5, exudate of Fremont cottonwood (*Populus fremontii* Wats.), UWO(PS)85-379.1 and UWO(PS)85-384.1, *Drosophila brooksae* Pipkin, Ganter et al. (1986); UWO(PS)91-127.2, exudate of red oak (*Quercus rubra* L.), Ontario, Canada.

Type strain: CBS 2745, isolated by Phaff et al. (1956), from *Drosophila pinicola.*

Comments: In nature, *K. wickerhamii* is confined to *Drosophila* species and to tree exudates that probably act as *Drosophila* habitats. Certain *K. wickerhamii* strains produce broad spectrum killer factors, effective not only against other *Kluyveromyces* and related species (Lehmann et al. 1987b, Vaughan-Martini and Rosini 1989), but also against a very wide array of unrelated yeasts (M.A. Lachance, unpublished results).

K. wickerhamii would easily be confused with other lactose-assimilating species of *Kluyveromyces* were it not for its inability to assimilate inulin and its resistance to 100 mg/l cycloheximide.

36.15. *Kluyveromyces yarrowii* van der Walt, E. Johannsen, Opperman & Halland (1986b)

Synonyms:

Torulopsis tannotolerans F.H. Jacob (1970)

Candida tannotolerans (F.H. Jacob) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Growth on YM agar: After 3 days at 25°C, the cells are spheroidal to ovoid, (3–6)×(4–9) μm, single, in pairs or in short chains. Growth is butyrous, glossy, and cream-colored.

Growth in glucose–yeast extract broth: A sediment and a partial ring are formed.

Dalmau plate culture on corn meal agar: Pseudomycelium is not formed.

Formation of ascospores: Ascus formation follows the conjugation of haploid cells of complementary mating types or asci may arise directly from stable diploid cells. One to four spheroidal ascospores are formed. The spores are liberated from the ascus soon after formation and tend to agglutinate.

The diploid strain CBS 8242 sporulates well after 2–5 days at 17–25°C on YM, malt extract, or McClary's acetate agar.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Inulin	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	ws
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
Cadaverine	–	Cycloheximide 100 mg/l	–
L-Lysine	–	Amino acid-free	+
Ethylamine·HCl	–	Growth at 37°C	–
50% Glucose	–		

Co-Q: 6 (Y. Yamada in: van der Walt et al. 1986b).

Mol% G+C: 34.5 (Stenderup et al. 1972).

Origin of the strains studied: CBS 2684, type strain of *Candida tannotolerans* and CBS 6070, both from tanning liquors containing tree bark, Jacob, France; CBS 8242, a diploid strain.

Complementary mating types: CBS 2684 and CBS 6070.

Type strain: CBS 8242, a stable diploid obtained by crossing auxotrophic subcultures of CBS 2684 (α) and CBS 6070 (a) (van der Walt et al. 1986b).

Comments: *Candida tannotolerans* was isolated from tanning liquors by Jacob (1970). After Yarrow observed ascospore formation in mixtures of strains of *Candida tannotolerans,* van der Walt et al. (1986b) described the teleomorph as *K. yarrowii*. This yeast is very similar in DNA base composition and physiological profile to *K. africanus* and *K. phaffii.* Unlike these, however, *K. yarrowii* is heterothallic and forms spherical ascospores.

The designation of an artificial diploid strain created from two mutagenenized parents as the type strain of a species may be questionable as a biological practice, but it follows the rules of the International Code of Botanical Nomenclature (Greuter et al. 1988) and must be accepted. This is all the more unfortunate because the diploid construct exhibits a certain amount of hybrid vigor, whereby growth on ethanol, glycerol, xylitol, glucono-δ-lactone, 10 mg/l cycloheximide, and YM agar with 40 mM HCl (the last four are not standard tests used in this chapter) was noticeably stronger for the diploid than for either haploid strain.

The liquors from which strains of *K. yarrowii* were isolated contained approximately 6% tannins extracted from the bark of various trees (Jacob 1970). Whether or not this is significant in defining the niche of this yeast remains to be seen. The 3 strains examined grew well on yeast nitrogen base agar supplemented with 0.5% tannic acid as the carbon source, which is not particularly unusual. Several other yeasts, including most *Kluyveromyces* species, grow on that medium.

Physiologically, *K. yarrowii* may be distinguished from other ethylamine-negative, four-spored *Kluyveromyces* species by its absence of growth on β-fructosides, 2-keto-D-gluconate, D-gluconate, and at 37°C.

Comments on the genus

The history of the taxonomy of *Kluyveromyces* is characterized by a series of interesting developments from the creation of the genus, prompted by the discovery

of *K. polysporus* (van der Walt 1956a), to the later inclusion (van der Walt 1965c) of several species at one time considered members of *Saccharomyces sensu lato* (van der Walt 1970d). The reader is referred to van der Walt's treatment (1970c) for details up to that point. Here, the discussion will emphasize the most recent reassessments of the boundaries of the genus based on phenetic, genetic, or molecular approaches, which are not without a number of fascinating peripeties. To facilitate the discussion, the genus is conveniently divided into three groups each representing species known to exhibit a number of properties in common (see Tables 27 and 28). Group A comprises *K. africanus, K. bacillisporus, K. blattae, K. delphensis, K. lodderae, K. phaffii, K. polysporus,* and *K. yarrowii.* Group B is made up of *K. aestuarii, K. dobzhanskii, K. lactis, K. marxianus,* and *K. wickerhamii.* Group C comprises *K. thermotolerans* and *K. waltii.*

Wickerham and Burton (1956a,b) divided species now considered as members of the genus *Kluyveromyces* with regard to sexual compatibility and ascospore shape, suggesting that they had evolved along two "lines of development". Van der Walt (1970c) extended this idea further, viewing the nutritionally specialized reniform spored species that produce four spores as primitive progenitors from which emerged more advanced species characterized either by the formation of larger ascospore numbers, or the acquisition of β-glucosidase, β-galactosidase or α-glucosidase activities. Another line of development, in which ascospores are spheroidal instead of reniform, was presumed to have undergone an analogous nutritional radiation. Ascospore shape was, in those cases, given considerable importance. Kock et al. (1988) reviewed this theme and extended it in the light of published molecular comparisons. In addition to placing much less emphasis on ascospore morphology, they reached the conclusion that ancestral members of the genus were in fact those that produce abundant pseudomycelium, assimilate a wider spectrum of nutrients, produce certain specific fatty acids, and possess diverse ascospore morphologies.

Because of the widespread ability of many *Kluyveromyces* species to be self-fertile (homothallic), a classical concept of biological species based on mating compatibility alone would be difficult to formulate. Hybridization between members of various taxa, including *K. dobzhanskii* and species now regarded as synonyms of *K. marxianus* or *K. lactis* was subjected to diverse interpretations and generated a fair amount of controversy. Wickerham and Burton (1956a,b) obtained interspecific hybrids of these yeasts. Bicknell and Douglas (1970) later showed that hybrids involving *K. marxianus* (syn. *K. fragilis*) and *K. lactis* or *K. dobzhanskii* were in fact allopolyploids. The potential for limited gene flow was demonstrated (Johannsen 1980) among all taxa later regarded by van der Walt and Johannsen (1984) as varieties of the single species, *K. marxianus*, as well as with *K. wickerhamii.* Limited hybridization was also reported between these and *K. waltii* or *K. thermotolerans.* Gene flow between species is not entirely surprising. It serves as a mechanism by which new species may arise, and thus accounts for reticulate evolution. The significance of interspecific hybridization involving *K. thermotolerans* was examined by Vaughan-Martini et al. (1987), who studied a single-spore isolate previously obtained by mass-mating of *K. marxianus* and *K. thermotolerans* (Johannsen and van der Walt 1978). The putative recombinant was phenotypically and genetically unstable, and could be made to revert to one parental state, that of *K. marxianus.* The significance of the results was complicated by the observation that the strain used to construct this hybrid may not have been an authentic representative of *K. thermotolerans.* Interspecific hybridization was most recently addressed by Steensma et al. (1988) whose results leave no doubt that *K. marxianus* and *K. lactis* represent different biological species, in spite of their propensity towards forming sporulating hybrids. One very obvious conclusion is wanting to be drawn regarding mating compatibility in the group B *Kluyveromyces* species: given that those yeasts have diverged into at least 5 fully distinct species and yet have conserved almost unaltered their sexual compatibility, it follows that the various gene products involved in this mating system must be extremely efficient at performing their function. A last thought on the difficulty of assessing the taxonomic significance of hybridization can be drawn from the following study. Witte et al. (1989) have obtained, by protoplast fusion, hybrids between *K. marxianus* and *Saccharomyces cerevisiae,* and between *K. thermotolerans* and *S. cerevisiae.* No one would argue that these could be anything but interspecific crosses. Electrokaryotypes of the hybrids demonstrated the presence of hybrid chromosomes, illustrating that once the hurdle of pre-zygotic isolation is sidestepped, speciation does not necessarily provide a complete barrier to gene recombination. While this does not ease our task of identifying species boundaries, it does urge us to exercise caution in interpreting out-of-context data that we deem taxonomically significant.

Because all the factors contributing to genetic isolation of yeast populations cannot be assessed in every case, the question of species delimitation is advantageously examined ex post facto, by measuring genetic divergence. The question of species delineation in *Kluyveromyces* has been addressed extensively by such methods. Nucleic acid reassociations will be considered first.

The molecular taxonomy of *Kluyveromyces* was pioneered by Bicknell and Douglas (1970), who obtained high degrees of heteroduplex formation between the non-repetitive DNAs of *K. marxianus* and the former taxon *K. fragilis,* hinting on their conspecificity. *K. dobzhanskii* and *K. wickerhamii* were found to be genetically distinct from *K. marxianus* and *K. lactis,* and the last two were recognized as distinct from each other. Based on rRNA–DNA duplex formation, these four taxa were shown, in addition, to have speciated fairly recently. The rRNA relatedness

of *K. delphensis* or *K. thermotolerans* to *K. lactis* was comparable to the relatedness of members of the genera *Saccharomyces, Torulaspora,* or *Zygosaccharomyces* to *K. lactis*. When compared to *Saccharomyces cerevisiae,* those *Kluyveromyces* species appeared heterogeneous in their rRNAs, but always distinct from *Saccharomyces, Torulaspora,* or *Zygosaccharomyces.* Martini (1973) confirmed that *K. marxianus, K. lactis, K. wickerhamii, K. thermotolerans, K. dobzhanskii,* and *K. delphensis* were genetically disjoint as assessed by heterologous DNA renaturation rates. In addition, he compared many pairs of DNAs from every *Kluyveromyces* species sensu van der Walt (1970c), setting the foundation for our current understanding of genetic relatedness in the genus. Fiol and Poncet (1980) performed DNA reassociations on certain strains. Their reassociation values were not in general agreement with those already in the literature, or with preliminary results to be published later by other workers. Specifically, they found relatively high amounts of reassociation between *K. marxianus* and *K. africanus, K. phaffii, K. dobzhanskii,* or *K. wickerhamii.* A likely interpretation of their findings is that the DNA used as tracer may have been enriched for certain conserved sequences, possibly ribosomal DNA, thus extending the useful range of DNA reassociation beyond the usual level of closely related species. The recent DNA reassociation studies of *Kluyveromyces* by Fuson et al. (1987) and Vaughan-Martini and Martini (1987b) are altogether sufficiently exhaustive to resolve most uncertainties about species boundaries. Lachance et al. (1993) ascertained the species status of *K. bacillisporus* by DNA reassociation with their putative nearest relatives among group A species. Taken all together, the results of these studies are consistent with the classification presented here.

Many authors have contributed to the determination of DNA base composition in *Kluyveromyces* (Bicknell and Douglas 1970, Nakase and Komagata 1971e, Martini et al. 1972, Poncet and Fiol 1972, Stenderup et al. 1972, Fuson et al. 1987, Vaughan-Martini and Martini 1987b, Lachance et al. 1993). While a broad agreement exists between data from different laboratories, a few exceptions should be noted and taken into account when making comparisons. The values of Poncet and Fiol (1972) tended to be lower than others, except for *K. polysporus,* for which the lowest value (33%) was from Nakase and Komagata (1971e). Because the publication of Fuson et al. (1987) is the most comprehensive and reports data uniformly based on the buoyant density method, only those values have been included in this chapter, with the exception of the value for *K. bacillisporus.* Base composition values are consistent with the separation of the genus *Kluyveromyces* into three natural groups. Group A species range from 34 to 40, group B species from 40 to 43, and group C species both have 46 mol% G + C.

Other molecular approaches have focused on patterns of protein or nucleic acid divergence assessed by various indirect methods. Lachance and Phaff (1979) clarified the species boundaries in group B species through an immunological comparison of β-glucanases. They showed that *K. marxianus, K. fragilis, K. bulgaricus, K. cicerisporus,* and *K. wikenii* should be considered synonyms, and that *K. lactis, K. vanudenii, K. drosophilarum,* and *K. phaseolosporus* were also probably conspecific, in spite of their heterogeneity of ascospore morphology. Sidenberg and Lachance (1983, 1986) pursued the question by means of isoenzyme electrophoresis. They confirmed the patterns elicited by DNA reassociation, concluding that introgression, if present among *Kluyveromyces* species shown to mate at high frequencies, had in fact been sufficiently rare to allow them to maintain separate gene pools. *K. thermotolerans* and *K. waltii* were clearly identified as distinct from each other and from group B species. Their ability to undergo some form of rare genetic interaction with other *Kluyveromyces* species must be considered a vestige of their evolutionary history, and not evidence that they represent "incipient species" (Kock et al. 1988). Restriction endonuclease mapping of the ribosomal DNA clusters of the type strains of *Kluyveromyces* (Lachance 1989) confirmed the cohesive nature of group B species, all of which shared some synapomorphic restriction sites with other members of the group. Group A species, not surprisingly, were less cohesive, but their respective rDNAs each shared some synapomorphic sites with other members of the group. Our inexperience with the significance of the variation in size and numbers of yeast chromosomes renders difficult the task of evaluating electrokaryography and its application to the systematics of *Kluyveromyces.* The available data (Kock et al. 1988, Sor and Fukuhara 1989) have usually confirmed the species boundaries proposed here, but have not provided new insight on their interrelatedness. Restriction analysis of mitochondrial DNA (Ragnini and Fukuhara 1988) revealed a great deal of polymorphism among the mitochondrial genomes of *Kluyveromyces* species. Particularly interesting was the observation that mtDNAs of most group B species contained an unusually high number of *Sac*II (CCGCGG) and *Hpa*II (CCGG) restriction sites. *K. marxianus* (five strains of *K. marxianus sensu stricto* and three strains of the synonym *K. bulgaricus*), group C species, and most group A species had few or no such sites. *K. aestuarii, K. africanus, K. delphensis, K. lodderae* occupied an intermediate position, with many *Hpa*II sites, but few, if any *Sac*II sites. Again, these data support the distinct status of *K. marxianus* and *K. lactis,* and the complex inter-relationships linking species in groups A, B, and C.

The poor success of numerical taxonomy in recognizing natural groups among yeasts probably derives from our inability to measure many genetically independent phenotypes in them. On the basis of cluster analysis, Campbell (1972) proposed the transfer of all *Kluyveromyces* species to *Saccharomyces* (*sensu* van der Walt 1970d), which has not met with much agreement in the taxonomic community. The physiological profiles of all ascogenous

yeasts listed in Barnett et al. (1990) have been analyzed by a similar method (M.A. Lachance, unpublished results) and the analysis has been used here to assess possible relationships and phenetic homogeneity of taxa. Taxa thought to be monophyletic on the basis of genetic or molecular data rarely arranged themselves into phylogenetically consistent patterns. Although intergeneric relationships may not be attainable by such methods, an ordination analysis performed by Poncet (1973b) on *Kluyveromyces* species gave results that were remarkably consistent with those of molecular studies conducted years after the publication of her paper. She identified the existence of groups A and B, and the distinct nature of *K. thermotolerans* (group C; *K. waltii* was not known at that time). Within group B, she correctly recognized the conspecificity of taxa now known to be synonyms of *K. marxianus,* and their independence from taxa known to be synonyms of *K. lactis.*

A number of novel properties thought to be potentially useful in the taxonomy of the genus *Kluyveromyces* have been examined. Gorin and Spencer (1970) examined proton magnetic resonance spectra of outer cell wall components. Their analyses properly identified kinships between *K. fragilis* and *K. marxianus,* and between *K. lactis* and *K. drosophilarum,* but also suggested significant degrees of relatedness between *K. thermotolerans* and *K. delphensis, K. polysporus* and *K. aestuarii,* and could not elicit any particular resemblance between *K. phaseolosporus* and other synonyms of *K. lactis.* Also targeting outer wall surface components, Campbell (1972) reported on antigenic characterizations of whole cells. Four antigenic classes were identified. Three antigenic determinants (A, B, and C) had little, if any predictive value with respect to phyletic relatedness. A fourth antigen (D) was unique to three species in group A, namely *K. delphensis, K. polysporus* and *K. lodderae.* Fiol and Claisse (1982) studied the spectrophotometric properties of whole cell cytochromes. The low temperature spectra were purported to separate *Kluyveromyces* groups A and B, and within group B, to elicit *K. aestuarii* and *K. wickerhamii* as distinct from the rest. Furthermore, groups A and B were said probably to constitute a monophyletic entity. Although these conclusions are in general agreement with the views presented here, it is not at all clear how they were arrived at on the basis of the data presented by those authors. Lehmann et al. (1987b) and Vaughan-Martini and Rosini (1989) demonstrated killer activity in some strains of every group B species, and in strains of *K. lodderae* and *K. phaffii.* The toxins are effective against yeasts from other genera as well as species of *Kluyveromyces.* Reciprocal killing is even possible (Lehmann et al. 1987b). Both studies indicated that killer patterns might be useful in biotype identification, but Vaughan-Martini and Rosini (1989) reached the general conclusion that killer patterns had little value in delineating species. Kock et al. (1988) found a correlation between long-chain fatty acid distribution in *Kluyveromyces* species and their broad evolutionary affinities as understood by most students of the genus. In particular, group B and C species were found to differ from most group A species by the presence of linoleic and linolenic acids among their long-chain fatty acids. The exception was *K. yarrowii,* which contains both acids, and *K. africanus,* which contains linoleic acid only. Perhaps inspired by the work of Carvalho-Silva and Spencer-Martins (1990) on the significance of lactose proton symports in *K. marxianus* and relatives, Kilian and coworkers (1991) surveyed the distribution of symports for five sugars in a strain of every *Kluyveromyces* species. Evidence that transport patterns are in good agreement with natural affinities is difficult to bring into focus, given that, in terms of symports, *K. lodderae* and *K. yarrowii* clustered closer to *K. marxianus* and *K. dobzhanskii* than to other group A species, and three synonyms of *K. lactis* clustered closer to group A than to *K. lactis* var. *drosophilarum.* The most unexpected result was the presence of four symports in *K. yarrowii* and the absence of symports in *K. phaffii,* in sharp contrast with the very similar physiologies of these two species. Last, a correlation is claimed between the number of symports and the number of compounds utilized. Careful examination of the data (Fig. 2 in Kilian et al. 1991) shows that the postulated trend arose to a large part out of errors made in rearranging the carbon source utilization profiles from a diagram taken from Kock et al. (1988).

Naumov (1987b) emended the genus *Zygofabospora* Kudryavtsev to accommodate certain species of *Kluyveromyces*, proposing that the latter name should apply only to the multispored species, and that possibly a separate genus should be erected for *K. phaffii* because of its diplontic life cycle. The rationale for this proposed reorganization (Naumov 1986a) was that the original descriptions of *Kluyveromyces* and *Zygofabospora* were clearly distinct, and that many species currently assigned to *Kluyveromyces* apparently fit the description of *Zygofabospora* better. When van der Walt (1965c) chose to combine multispored *Kluyveromyces* species together with others in a single genus, he did so on the basis that ascus deliquescence correlated better than spore number with other characteristics of the species, and thus was a better basis for defining a genus monothetically. By contrast, the two major genera created by Naumov's proposals would be clearly polyphyletic. Indeed, *K. lodderae* and *K. delphensis,* which are proposed members of *Zygofabospora,* bear considerably less resemblance to *K. marxianus, K. lactis,* or other putative *"Zygofabospora"* species than to *K. phaffii* or to the multispored, "true" *Kluyveromyces* species. By many criteria (Poncet 1973b, Kock et al. 1988), Group A species are phenetically homogeneous. The most recently discovered species, *K. bacillisporus,* is diplontic, like *K. phaffii,* but also fits well within group A, which serves as additional evidence against splitting group A species. Until the phylogenetic affinities of group C species are clarified, it is not unreasonable

to think that *Kluyveromyces*, as currently defined, might be monophyletic. Alternatively, group C species might be classified better elsewhere, but evidence of polyphyly in *Kluyveromyces* is simply lacking, and their transfer at this point would be unjustified. A fortiori, the reassignment of these and other *Kluyveromyces* species into one monotypic and two polyphyletic genera is to be rejected outright.

Morikawa et al. (1985) reported the isolation of a new yeast species capable of fermenting D-xylose to relatively large amounts (3%) of ethanol. The yeast reportedly resembled *K. lactis* in its assimilation patterns, and was provisionally named *K. cellobiovorus* pending a formal description. Strain CBS 7153 was examined and ascospores were not found. Unlike any *Kluyveromyces* species, this yeast assimilates *N*-acetyl-D-glucosamine, ethyl acetate, and hexadecane vigorously, utilizes D-glucosamine slowly but strongly, and exhibits very strong and rapid growth in YM agar supplemented with 40 mM hydrochloric acid. Strain CBS 7153 was studied by means of rDNA restriction mapping (Lachance 1989), electrokaryotyping (Sor and Fukuhara 1989), and killer sensitivity patterns (Vaughan-Martini and Rosini 1989). In all cases it was clear that the strain cannot be assigned to the genus *Kluyveromyces*. In fact, Martini and Vaughan-Martini (1992) showed, by means of DNA reassociation, a high degree of genetic similarity between strain CBS 7153 and the type culture of *Candida intermedia*, a Q-9 species thought by some so be related to the genus *Debaryomyces*.

Weber et al. (1992) recently described two soil isolates as *K. piceae*, reported to differ from other group A species by the utilization of ethylamine and lysine as nitrogen sources, and galactitol as carbon source. DNA reassociation suggested that these strains represent a distinct species, possibly related to *K. lodderae*. Strains CBS 7738 and CBS 7739 were examined both in this laboratory and at the Centraalbureau voor Schimmelcultures (D. Yarrow, personal communication). Neither strain utilized the three aforementioned compounds. Although the two strains appeared strikingly similar in many ways, the second strain (type) had apparently lost the ability to sporulate or to grow on yeast nitrogen base agar with any simple carbon source (including glucose). Scant growth was observed after supplementing the medium with leucine. In view of this perplexing instability, it appears advisable to defer recognition of this species until these problems are clarified.

37. *Lipomyces* Lodder & Kreger-van Rij

M.Th. Smith

Diagnosis of the genus

Cells are ellipsoidal or globose, reproducing by multilateral budding. Oval to cylindrical giant cells may occur. Pseudomycelium is absent. Cells of most strains are surrounded by a thick mucoid capsule. Most strains form starch-like polysaccharides in this capsule. Cells in older cultures usually include a large fat globule.

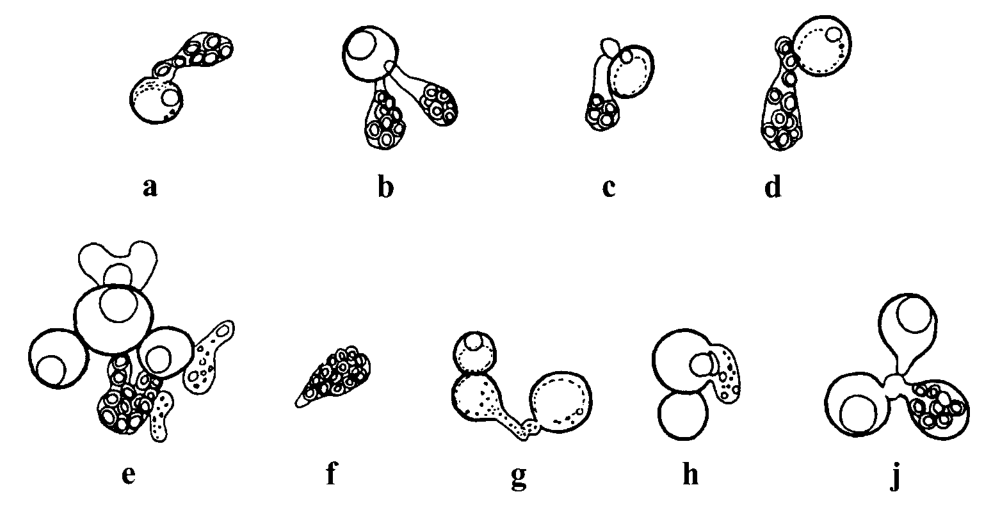


Fig. 107. *L. starkeyi*, CBS 1807. Various ways of conjugation and of formation of asci (see text) (Slooff 1970a).

Asci develop in various ways; an "active bud" is most frequently involved in formation of the ascus. This may or may not be preceded by a discernable conjugation.

(1) When the asci develop from active buds only, they often enlarge to an elongate pear shape (Fig. 107a,c,d). The wall of this type of ascus is thin. Two asci or more may arise from one parent cell (Fig. 107b). The entire ascus may liberate itself from the cell when mature (Fig. 107f). The number of spores may be up to 16 or more per ascus; in some species the number of spores is eight to four or less per ascus.

(2) When the ascus is the result of conjugation of an active bud and a protuberance of a cell, the ascus wall is thicker (Fig. 107g, j). Complicated groups of these cell–bud conjugations are frequently present on sporulation media.

(3) A parent cell, after having produced an ascus through an active bud, may itself turn into an ascus. Fully grown cells containing ascospores have been observed. These asci possess thick walls.

Ascospores are globose, ellipsoidal to oblong-ellipsoidal, generally liberated at maturity. The pigmented walls are of a light amber to brown color. The ascospore wall may be warty, smooth, or it bears ridges running longitudinally on the surface.

Germination of ascospores is by swelling to the size of a small cell followed by budding. Conjugation of swollen spores has been observed.

Fermentation is absent. Nitrate is not assimilated. Diazonium blue B reaction is negative.

Type species

Lipomyces starkeyi Lodder & Kreger-van Rij

Species accepted

1. *Lipomyces japonicus* van der Walt, M.Th. Smith, Y. Yamada & Nakase (1989)
2. *Lipomyces kononenkoae* Nieuwdorp, Bos & Slooff (1974)
   a. *Lipomyces kononenkoae* Nieuwdorp, Bos & Slooff subspecies[1] *kononenkoae* (1995)
   b. *Lipomyces kononenkoae* subspecies *spencer-martinsiae* van der Walt & M.Th.Smith (1995)
3. *Lipomyces lipofer* Lodder & Kreger-van Rij ex Slooff (1970)
4. *Lipomyces starkeyi* Lodder & Kreger-van Rij (1952)
5. *Lipomyces tetrasporus* Nieuwdorp, Bos & Slooff (1974)

[1] Editor's note: In this book the extent of genetic divergence between subspecies and between varieties of a species is considered comparable.

Key to species

See Table 29.

1. a Melibiose assimilated → 2
   b Melibiose not assimilated .... *L. japonicus*: p. 249
2(1). a Growth at 40°C positive .... *L. kononenkoae* ssp. *spencer-martinsiae*: p. 250
   b Growth at 40°C negative → 3
3(2). a Ascospores with ridges in light- and transmission electron microscopy .... *L. tetrasporus*: p. 252
   b Ascospores without ridges → 4
4(3). a Ascospores warty .... *L. starkeyi*: p. 252
   b Ascospores smooth → 5
5(4). a Erythritol and butane-2, 3-diol assimilated .... *L. lipofer*: p. 251
   b Erythritol and butane-2, 3-diol not assimilated .... *L. kononenkoae* ssp. *kononenkoae*: p. 250

Table 29
Key characters of species in the genus *Lipomyces*

Species	Assimilation			Growth at 40°C	Ascospore wall
	Melibiose	Erythritol	Butane 2, 3 diol		
Lipomyces japonicus	–	–	+	–	warty
L. kononenkoae ssp. *kononenkoae*	+	–	–	–	smooth
L. kononenkoae ssp. *spencer-martinsiae*	+	–	–	+	smooth
L. lipofer	+	+	+	–	smooth
L. starkeyi	+	v	v	–	warty
L. tetrasporus	+	+	+	–	ridges

Systematic discussion of the species

37.1. *Lipomyces japonicus* van der Walt, M.Th. Smith, Y. Yamada & Nakase (van der Walt et al. 1989c)

Synonym:
Smithiozyma japonica (van der Walt, M.Th. Smith, Y. Yamada & Nakase) Kock, van der Walt & Y. Yamada (1995)

Growth in malt extract: After 3 days at 25°C, cells are globose, 3.5–7.0 μm in diameter, rarely subglobose, ellipsoidal or ovoidal, encapsulated, contain lipid globules, and occur singly or in pairs. Growth is scant. After one month, a sediment and an incomplete ring are formed.

Growth on malt agar: After 3 days at 25°C, the cells have the same appearance as observed in malt extract. The streak culture is watery to viscous, creamish-opaque, glistening, and smooth with an entire margin.

Dalmau plate culture on corn meal agar: After 10 days at 25°C, neither hyphae nor pseudohyphae are formed.

Formation of ascospores: Asci are saccate, (7.0–13.0×3.0–6.0) μm, persistent, as a rule attached, with 1–4 spores, and arise from the fusion of protuberances of adjacent or non-adjacent cells, or from enlarging protuberances of single cells, or infrequently by automixis. Ascospores are globose, (2.0–3.5) μm, warty, amber or brown when mature, and contain a lipid globule. Actively sporulating cultures turn brown.

Sporulation was observed on 2% malt- and V8 agars at 18°C, after 7–14 days.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Butane 2, 3 diol	+	Galactonate	v
Starch formation	+	Growth at 30°C	+
0.1% Cycloheximide	+	Growth at 35°C	–

Co-Q: 9 (van der Walt et al. 1989c).

Mol% G+C: 41.5–42.2, CBS 7319, CBS 7549, CBS 7550 (T_m: Smith et al. 1995a).

Origin of the strains studied: CBS 7319 (NRRL Y-17357), garden soil, Kanagawa Prefecture, Japan, J.P. van der Walt; CBS 7549, uncultivated soil, Bronkhorstspruit District, Transvaal, South Africa, E.L. Jansen van Rensburg; CBS 7550, uncultivated soil, Frankfort, Orange Free State, South Africa, E.L. Jansen van Rensburg.

Type strain: CBS 7319 (NRRL Y-17357), isolated by J.P. van der Walt.

37.2. *Lipomyces kononenkoae* Nieuwdorp, Bos & Slooff (1974)

This species has two subspecies:

***Lipomyces kononenkoae* Nieuwdorp, Bos & Slooff ssp. *kononenkoae* (1995)**

***Lipomyces kononenkoae* ssp. *spencer-martinsiae* van der Walt & M.Th. Smith (Smith et al. 1995a)**

Growth in malt extract: After 7 days at 25°C, the cells are predominately globose, 5.0–8.5 µm in diameter, or rarely ellipsoidal, (4–5.8×5.5–8.5) µm. The cell walls and contents are faintly refractile, except those of the larger cells. Only a few small oil globules are present. After one month a sediment, a ring, and occasionally a thin, moist pellicle are present.

Growth on malt agar: After 7 days at 25°C, cells are mostly globose, 4–10 µm in diameter, or rarely ellipsoidal, (4.0–6.0×6.2–8.0) µm. Some cells produce a single bud. After one month, the streak culture is creamy-white, glistening and mucoid.

Dalmau plate culture on corn meal agar: Neither hyphae, nor pseudohyphae are formed.

Formation of ascospores: Conjugation of two "active" buds (Slooff 1970a), initiated on one parent cell, frequently precedes the formation of very thin-walled asci. Globose spores are formed and free spores often lie in a cluster in close contact with the parent cell (Fig. 108). Pear-shaped asci also occur, possibly arising from diploidized single buds. Two or more asci may occur on one parent cell. YM agar stimulates the development of protuberances; fully grown cells with protuberances may be transformed directly into asci. The usual number of spores per ascus is 8, but varies from 2 to 16.

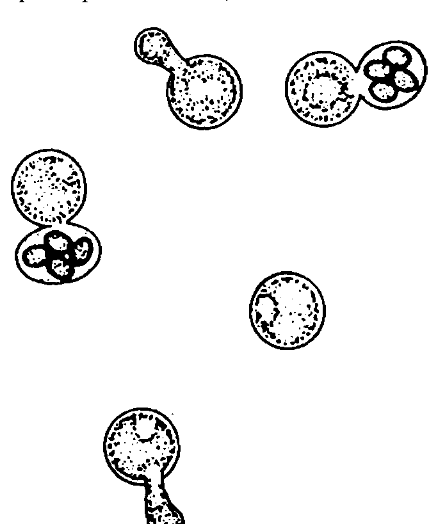


Fig. 108. *L. kononenkoae* ssp. *kononenkoae*, CBS 2514 (Phaff and Kurtzman 1984). Globose spores within asci.

Sporulation was observed on corn meal-, 1/10 V8, YM and 1/10 YM agars after 7 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	–
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	+	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	v	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

Starch formation	–	Growth at 30°C	+
0.1% Cycloheximide	+	Growth at 37°C	v
Galactonate	v	Growth at 40°C	–

Co-Q: 9 (Yamada et al. 1986c, Billon-Grand 1987).

Mol% G+C: 48.3, CBS 2514 (BD: Phaff and Holzschu, in Phaff and Kurtzman 1984); 47.0–48.0, CBS 2514, CBS 7535, CBS 8113, CBS 8114 (T_m: Smith et al. 1995a).

Supplementary description of *L. kononenkoae* ssp. *spencer-martinsiae*: The subspecies *spencer-martinsiae* differs from the subspecies *kononenkoae* by its failure to assimilate D-xylose, galactitol and succinic acid. The assimilation of trehalose and ethanol is variable, and of glycerol is positive. The subspecies is able to grow at 40°C.

Origin of the strains of ssp. *kononenkoae* studied: CBS 2514 (IFO 10375, NRRL Y-11553), soil of citrus orchard, Los Lomas, Trinidad, J.H. Becking; CBS 7535, soil, Magoeba's Kloof, Transvaal, South Africa, J.P. van der Walt; CBS 8113, CBS 8114, soil, South Africa, J.P. van der Walt.

Type strain: CBS 2514, isolated by J.H. Becking.

Origin of the strains of ssp. *spencer-martinsiae* studied: CBS 5608 (ATCC 44833; NRRL Y-11554), soil, Nigeria, E. Drouhet; CBS 7534, soil, Pretoria district, Transvaal, South Africa, J.P. van der Walt; CBS 7543, soil, Kwa-Mbonambi State Forest Reserve, Natal, South Africa, J.P. van der Walt; CBS 7681, CBS 7682, soil, Bronkhorstspruit District, Transvaal, South Africa, J.P. van der Walt.

Type strain: CBS 5608, isolated by E. Drouhet.

Comments: In a study of species delimitation in the genus *Lipomyces* by genome comparisons (Smith et al. 1995a), it was shown that strains assigned to *L. kononenkoae* comprised two genetically discernable

entities characterized by an average nDNA relatedness of 47%. The heterogeneity of *L. kononenkoae* was first observed by Hossack and Spencer-Martins (1978) and Spencer-Martins (1983) who noted differences in lipid composition, amylolytic complexes, optimal growth temperature and utilization of β-cyclodextrin and D-deoxyglucose. Since the two taxa are phenotypically distinguishable, Smith et al. (1995a) proposed to subdivide *L. kononenkoae* into subspecies.

37.3. *Lipomyces lipofer* Lodder & Kreger-van Rij ex Slooff (1970a)

Synonyms:

Torula lipofera den Dooren de Jong (1927)
Torulopsis lipofera (den Dooren de Jong) Lodder (1934)
Cryptococcus lipoferus (den Dooren de Jong) Skinner (1947b)
Waltomyces lipofer (Lodder & Kreger-van Rij ex Slooff) Y. Yamada & Nakase (1985)

Growth in malt extract: After 7 days at 25°C, growth is scant. The cells are granular, sometimes vacuolated, and globose to ellipsoidal, (3.2–8.0×5.0–12.0) μm. Walls and cells are faintly refractile, and sometimes contain a few small lipid globules. A thin ring may be present. After one month, some sediment forms and a few slimy islets may be present.

Growth on malt agar: After 7 days at 25°C, the cells are globose to ellipsoidal (4.0–8.0×5.0–14.0) μm, and contain vacuoles and small lipid granules. Large spheroidal cells that contain one large lipid globule may occur. After one month, the streak culture is white to cream-colored, glistening and pasty or mucoid.

Dalmau plate cultures on corn meal agar: Neither hyphae nor pseudohyphae are formed.

Formation of ascospores: Active buds that are formed on lipid-containing cells become transformed into asci as follows (Fig. 109):

(1) An active bud enlarges and the contents turn granular and divide into spores. The number of spores is 4 to 20, but sometimes less than 4.

(2) Two active buds on one cell fuse; the fusion product enlarges and develops into an ascus. The number of spores is 3 to 16.

(3) Two active buds on separate cells or on neighboring cells copulate; this case is infrequently observed in *L. lipofer.*

(4) An active bud, growing lengthwise, fuses with the conjugation tube of another cell; the two cells joined by a conjugation tube turn into one ascus. In this case the recipient cell frequently contains the greater part of the ascospores: 8–12 per ascus. Ascospores are ellipsoidal and contain an oil droplet. The color is olive-green to light amber. Malachite green is readily taken by the spores. The spore wall is smooth.

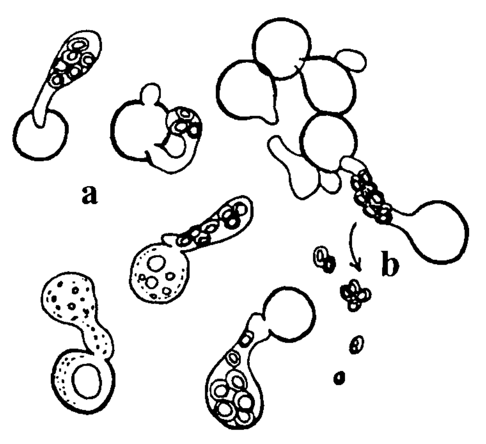


Fig. 109. *L. lipofer,* CBS 944. Asci with ascospores on Starkey's ethanol agar (Slooff 1970a).

Sporulation is observed on corn meal-, V8-, 1/10 V8, YM, 1/10 YM agars after one to two weeks.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+/w
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	v	Succinate	+/w
L-Arabinose	v	Citrate	+/w
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	s

Additional assimilation tests and other growth characteristics:

Butane 2,3 diol	+	Galactonate	+
Starch formation	+/w	Growth at 30°C	v
0.1% Cycloheximide	–	Growth at 35°C	–

Co-Q: 10 (Yamada et al. 1986c, Billon-Grand 1987).

Mol% G+C: 49.3, CBS 944 (BD: Phaff and Holzschu, in Phaff and Kurtzman 1984); 46.9–48.5, CBS 944, CBS 2513, CBS 5841, CBS 7603 (T_m: Smith et al. 1995a).

Origin of the strains studied: CBS 944 (ATCC 32031, IFO 1288, NRRL Y-2542), garden soil, Netherlands, L.E. den Dooren de Jong; CBS 2513 (IFO 10378, NRRL Y-11556), soil botanic garden, Leiden, Netherlands, J.H. Becking; CBS 5841 (ATCC 10742, IFO 10379, NRRL Y-1351), A.C. Thaysen; CBS 5842 (ATCC 32371, IFO 10380, NRRL Y-6333), soil, Wales, U.K., D. Jones; CBS 7602, cultivated podzolic soil, Biological Station of Perm University, Russia, I.P. Bab'eva; CBS 7603, cultivated meadow soil, Kirghiz, Armenia, I.P. Bab'eva.

Type strain: CBS 944, isolated by L.E. den Dooren de Jong.

Comments: *Lipomyces lipofer* is the only species in *Lipomyces* with the coenzyme Q-10 system. On the basis of this chemotaxonomic character, as well as ultrastructural differences in ascospore morphology (Smith and Batenburg-van der Vegte 1984), Yamada and Nakase (1985) transferred this species to the new genus *Waltomyces*. However, Kurtzman and Liu (1990) showed by molecular studies that *W. lipofer* and the remaining *Lipomyces* species are congeneric.

37.4. *Lipomyces starkeyi* Lodder & Kreger-van Rij (1952)

Growth in malt extract: After 7 days at 25°C, growth is scant. Cells are granular and some have vacuoles; lipid globules are scarce and small; cells are globose to ellipsoidal, (4–7×5–11) µm. Cell walls and contents are faintly refractile, except in some large globose cells and those cells that contain a single large lipid globule. A thin ring may be present. After one month, there are some small moist islets on the surface.

Growth on malt agar: After one week, the cells are similar as in malt extract, except that occasional large round cells with a single large lipid globule occur and the cells are only moderately granulated. After one month, the streak culture is white to light cream-colored, glistening, smooth, and very mucoid.

Dalmau plate culture on corn meal agar: Neither hyphae nor pseudohyphae are produced.

Formation of ascospores: Active buds developed by lipid-containing cells are transformed into asci by the same four processes as described for *L. lipofer.* The highest numbers of spores were found in asci derived from one active bud or from the fusion of two active buds (Fig. 107e,h) and may be 16 or more per ascus. The usual number in the type strain was 8 spores, but it may vary from 4–20. Other strains develop 2–6 spores per ascus, the usual number being 4. Starkeyi's strain number 72, which formerly sporulated with 1–4 spores per ascus, could no longer be induced to sporulate. In those cases where conjugation between bud and cell was involved, the number of spores did not exceed 8. Ascospores are ellipsoidal and contain an oil drop. The color is light amber to brown. Malachite green readily stains the spores. The spore wall is warty.

Sporulation was observed on Starkeyi's ethanol-, corn meal-, V8-, 1/10 V8-, YM-, 1/10 YM agars after two weeks.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	v	Erythritol	v
Cellobiose	v	Ribitol	v
Trehalose	v	Galactitol	v
Lactose	v	D-Mannitol	v
Melibiose	+	D-Glucitol	v
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	v
Inulin	v	D-Gluconate	v
Soluble starch	v	DL-Lactate	–
D-Xylose	v	Succinate	v
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	v
D-Ribose	v	Hexadecane	n
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	s

Additional assimilation tests and other growth characteristics:

Butane 2,3 diol	v	Growth at 30°C	+
Starch formation	v	Growth at 35°C	v
0.1% Cycloheximide	+	Growth at 40°C	–
Galactonate	v		

Co-Q: 9 (Yamada et al. 1986c, Billon-Grand 1987).

Mol% G+C: 48.1, CBS 1807 (BD: Phaff and Holzschu, in Phaff and Kurtzman 1984); 46.3–48.0, 6 strains, including CBS 1807, CBS 2512, CBS 7545, CBS 8064 (T_m: Smith et al. 1995a).

Origin of the strains studied: CBS 1807 (IFO 1289, NRRL Y-1388), soil, USA, R.L. Starkey; CBS 1809 (ATCC 64135, IFO 10382, NRRL Y-11558), dry mutant ex CBS 1807, R.L. Starkey; CBS 2512 (IFO 10383, NRRL Y-11559), soil, Wageningen, Netherlands, H.J. Becking; CBS 6047 (ATCC 64137, NRRL Y-11561), soil, Haren, Netherlands, N.J.W. Kreger-van Rij; CBS 7536, soil, Thornton, Canada, J.P. van der Walt; CBS 7537, soil, Botanic garden, Cape Town, South Africa, J.P. van der Walt; CBS 7544, CBS 7545, soil, Mount Sheba forest reserve, Transvaal, South Africa, J.P. van der Walt; CBS 8064, lemons, France, F. Seigle-Murandi; and 12 strains more, isolated from soil of various locations in South Africa.

Type strain: CBS 1807, isolated by R.L. Starkey.

37.5. *Lipomyces tetrasporus* Nieuwdorp, Bos & Slooff (1974)

Synonyms:

Zygolipomyces tetrasporus Krasil'nikov, Bab'eva & Meavadh (1967) nom. nud.

Zygolipomyces lactosus Krasil'nikov, Bab'eva & Meavadh (1967) nom. nud.

Growth in malt extract: After 7 days at 25°C, the cells are globose to short-ellipsoidal, (4–7×4–9) µm, and occur singly, in pairs or in small clusters. A thin ring may be present. After one month, there is usually a ring and sometimes a mucoid pellicle as well as a small amount of sediment.

Growth on malt agar: The cells are similar to those in malt extract. After one month, the streak culture is

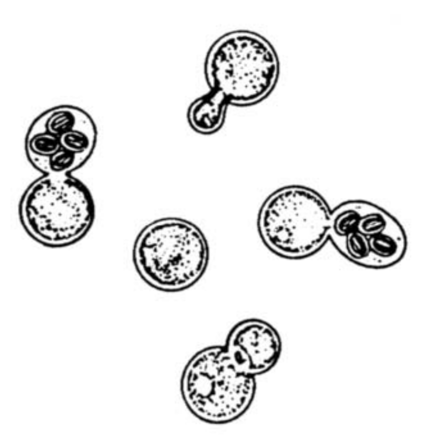


Fig. 110. *L. tetrasporus*, CBS 5910. Budding cells and asci with ascospores (Phaff and Kurtzman 1984).

white to light cream-colored, glistening, smooth, and very mucoid.

Dalmau plate culture on corn meal agar: Neither hyphae nor pseudohyphae are formed.

Formation of ascospores: The ascus develops from "active buds" or, more frequently, from a young cell conjugating by a tube with a bud from another cell. Often, fully grown cells develop ascospores without evident previous conjugation. There are 1–4 spores per ascus (Fig. 110). At maturity, the spores are released from the ascus. Ascospores are amber to brown and have lengthwise ridges.

Sporulation was observed on Starkeyi's ethanol-, corn meal-, YM-, 1/10 YM agars after two weeks.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	v	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	+	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	+	Citrate	w
D-Arabinose	+	Inositol	v
D-Ribose	v	Hexadecane	n
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	s

Additional assimilation tests and other growth characteristics:

Butane 2,3 diol	+	Galactonate	+
Starch formation	+/w	Growth at 35°C	v
0.1% Cycloheximide	+	Growth at 37°C	–

Co-Q: 9 (Yamada et al. 1986c, Billon-Grand 1987).

Mol% G+C: 49.1, CBS 5910 (BD: Phaff and Holzschu, in Phaff and Kurtzman 1984); 47.3–49.1, including CBS 5910, CBS 6051, CBS 6132, CBS 7656 (T_m: Smith et al. 1995a).

Origin of the strains studied: CBS 1808 (IFO 10367, NRRL Y-11564), soil, USA, R.L. Starkey; CBS 1810 (IFO 10388, NRRL Y-7038), soil, Ottawa, Canada, Harmsen; CBS 2511 (IFO 10389, NRRL Y-11565), soil, Monaco, J.H. Becking; CBS 5607 (IFO 10390), soil, Reunion, M. Dommerques; CBS 5910 (ATCC 32372, IFO 10391, NRRL Y-11562), soil, USSR, I.P. Bab'eva, type strain; CBS 5910.1 (IFO 10392), Schernozom soil, Orenburg district, USSR, I.P. Bab'eva; CBS 5911, 6049, 6050, 6051, soil, USSR, I.P. Bab'eva; and 9 strains more of soil from various locations.

Type strain: CBS 5910, isolated by I.P. Bab'eva.

Comments on the genus

Since the treatment of Phaff and Kurtzman in the last edition of "The Yeasts" (1984), the genus *Lipomyces* and related genera of the Lipomycetaceae have been the subject of many morphological, biochemical and molecular studies. Morphological examinations concerned the ultrastructure of ascospore walls and septa (Smith and Batenburg-van der Vegte 1984, Smith et al. 1995b). Biochemical studies were related to electrophoretic comparisons of enzymes (Yamazaki and Goto 1985, Yamada and Matsumoto 1988b), coenzyme Q systems (Yamada et al. 1986c, Billon-Grand 1987), carbohydrate profiles of whole-cell hydrolyzates (Weijman and van der Walt 1989), fatty acids and eicosanoids (Botha and Kock 1993, Cottrell et al. 1986, Cottrell and Kock 1989, Lomascola et al. 1994), electrophoretic karyotypes (Cottrell and Kock 1990), ferrichrome production (van der Walt et al. 1990a) and arachidonic acid metabolism (Kock et al. 1992). Molecular studies were focused on mtDNA analyses (Lodolo et al. 1990), ribosomal RNA sequence divergence (Kurtzman and Liu 1990, Yamada and Nogawa 1990b) and genome comparisons (Smith et al. 1995a).

The most relevant taxonomic proposals concerned two species. *Lipomyces anomalus* was transferred to the new genus *Babjevia* as *B. anomala* by Smith et al. (1995b) on the basis of molecular data from Kurtzman and Liu (1990) and Yamada and Nogawa (1990b), as well as because of the ultrastructure of septa and ascospore walls. *L. lipofer* was transferred to the new genus *Waltomyces* by Yamada and Nakase (1985) because it is the only species with coenzyme Q-10, a characteristic considered by some investigators to be important on the genus level. However, this transfer is not accepted in this treatment since Kurtzman and Liu (1990) showed *Waltomyces* and *Lipomyces* to be congeneric on basis of rRNA sequence divergence. For similar reasons, the proposed transfer of *L. japonicus* to *Smithiozyma* by Kock et al. (1995) has not been accepted.

38. *Lodderomyces* van der Walt

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. True hyphae are not produced, but pseudohyphae are present.

Asci are unconjugated, persistent, and produce one, or rarely two, ellipsoidal to elongate ascospores.

Glucose and certain other sugars are fermented. Nitrate is not assimilated. Pellicles are not formed on the surface of liquid media. Coenzyme Q-9 is produced. Diazonium blue B reaction is negative.

Type species

Lodderomyces elongisporus (Recca & Mrak) van der Walt

Species accepted

1. *Lodderomyces elongisporus* (Recca & Mrak) van der Walt (1971)

Systematic discussion of the species

38.1. *Lodderomyces elongisporus* (Recca & Mrak) van der Walt (1971)

Synonyms:

Saccharomyces elongisporus (as *S. elongasporus*) Recca & Mrak (1952)

Lodderomyces elongisporus (Recca & Mrak) van der Walt (1966a) nom. inval.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells may occasionally be spheroidal but they usually are ellipsoidal to elongate (2.6–6.3)×(4.0–7.4) µm, and occur singly, in pairs, or in small clusters. Growth is butyrous, faintly glistening, and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant well-branched pseudohyphae with blastoconidia. True hyphae are not formed. Aerobic growth is tannish-white, butyrous, smooth and glistening. Single colonies are low convex with a raised center. A faintly acidic odor is present.

Formation of ascospores: Asci are unconjugated, persistent, and transformed from vegetative cells. Each ascus forms one, rarely two, long-ellipsoid shaped ascospores (Fig. 111). Van der Walt (1984b) reported heat-treated ascosporogenous cultures to give only colonies that were ascosporogenous. However, it is not certain that this species is homothallic because the ploidy of the ascospores is unknown.

Ascospores were observed on V8 agar after 7–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+/w
Soluble starch	–	DL-Lactate	–
D-Xylose	+/w	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

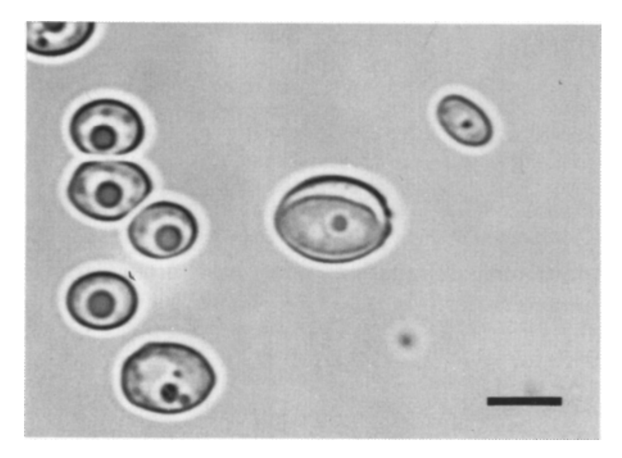


Fig. 111. *L. elongisporus*, CBS 2605. Unconjugated ascus with an ascospore, after 10 days on V8 agar at 25°C. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 2605 (Yamada et al. 1977).

Mol% G+C: 39.9, 39.5, CBS 2605, CBS 2606 (T_m: Meyer and Phaff 1969).

Origin of the strains studied: CBS 2605 (NRRL YB-4239), type strain of *Saccharomyces elongasporus,* from concentrated orange juice; CBS 2606 (NRRL YB-4240), soil, South Africa, from J.P. van der Walt; CBS 5912 (NRRL Y-7681), from a finger nail, Finland; CBS 6182 (NRRL Y-7682), from baby cream, Netherlands.

Type strain: CBS 2605, from concentrated orange juice.

Comments on the genus

Recca and Mrak (1952) described *Saccharomyces elongasporus* for a strain isolated from concentrated orange juice. Van der Walt (1966a) pointed out that *S. elongasporus* was unique among species of *Saccharomyces* because of its elongated ascospores and capability to assimilate higher paraffins such as decane and hexadecane. Because of these unique properties, van der Walt (1966a) described the genus *Lodderomyces* for *S. elongasporus*, and then later revalidated the genus (van der Walt 1971).

Because of phenotypic similarities, *L. elongisporus* had been considered the teleomorph of *Candida parapsilosis.* Meyer and Phaff (1972) demonstrated from comparisons of nDNA relatedness that the two taxa are separate species. Nakase et al. (1979) reported that isolates designated *C. parapsilosis* form II represented the anamorph of *L. elongisporus,* and the isozyme comparisons of Yamazaki and Komagata (1982a) supported this proposal. Hamajima et al. (1987) confirmed from nDNA complementarity that strains of *C. parapsilosis* form II represent the anamorph of *L. elongisporus.* Interspecific relationships among members of the *Lodderomyces* complex have been assessed from comparisons of 18S rRNA/rDNA sequences. Barns et al. (1991) showed a close relationship between *C. parapsilosis, C. tropicalis, C. viswanathii* and *C. albicans,* and James et al. (1994a) reported *L. elongisporus* to be closely related to *C. parapsilosis.*

39. *Metschnikowia* Kamienski

M.W. Miller and H.J. Phaff

Diagnosis of the genus

Vegetative reproduction is by multilateral budding. Cells are spheroidal to ellipsoidal, as well as pyriform, cylindroid, or lunate. Pseudomycelium is rudimentary, rarely lacking.

Asci are elongate: clavate, sphaeropedunculate or ellipsoidopedunculate. Ascospores are needle-shaped, attenuate at one or both ends, and without a whip-like appendage. One or two spores form per ascus, depending on the species.

Some species are parasitic in invertebrates, as well as free-living in aquatic habitats; others are terrestrial, free-living, and often associated with flowers.

Sugars are fermented by most species. Nitrate is not assimilated. Ubiquinone system is Co-Q 9. Diazonium blue B reaction is negative.

Type species

Metschnikowia bicuspidata (Metschnikoff) Kamienski

Species accepted

1. *Metschnikowia agaves* Lachance (1993)
2. *Metschnikowia australis* (Fell & I.L. Hunter) Mendonça-Hagler, Hagler, Phaff & Tredick (1985)
3. *Metschnikowia bicuspidata* (Metschnikoff) Kamienski (1899)
   a. *Metschnikowia bicuspidata* (Metschnikoff) Kamienski var. *bicuspidata* (1969)
   b. *Metschnikowia bicuspidata* var. *californica* Pitt & M.W. Miller (1970)
   c. *Metschnikowia bicuspidata* var. *chathamia* Fell & Pitt (1969)
4. *Metschnikowia gruessii* Giménez-Jurado (1992)
5. *Metschnikowia hawaiiensis* Lachance, Starmer & Phaff (1990)
6. *Metschnikowia krissii* (van Uden & Castelo-Branco) van Uden (1962)
7. *Metschnikowia lunata* Golubev (1977)
8. *Metschnikowia pulcherrima* Pitt & M.W. Miller (1968)
9. *Metschnikowia reukaufii* Pitt & M.W. Miller (1968)
10. *Metschnikowia zobellii* (van Uden & Castelo-Branco) van Uden (1962)

Key to species

See Table 30.

1. a Asci formed by differentiation of large, thick-walled refractile chlamydospores → 2
   b Asci formed from haploid or diploid vegetative cells → 4

2(1). a Asci formed from spheroidal cells ("pulcherrima" cells), asci sphaeropedunculate; pulcherrimin pigment usually produced → 3
   b Asci formed from ellipsoidal to cylindroidal cells ("reukaufii" cells), asci ellipsoidopedunculate to clavate; pulcherrimin pigment not produced → 5

3(2). a Vegetative cells predominantly lunate. Pulcherrimin pigment not produced .... *M. lunata*: p. 263
   b Vegetative cells globose to ellipsoidal. Pulcherrimin pigment produced by most strains in media with ferric ions .... *M. pulcherrima*: p. 264

4(1). a Asci clavate, containing a single ascospore, pointed at one end only → 6
   b Clavate asci arising from diploid cells, each containing two acicular ascospores .... *M. bicuspidata*: p. 259
   c Clavate asci arising from a zygote of two conjugating haploid cells of opposite mating type; two acicular spores per ascus → 7

5(2). a 2-Ketogluconate assimilated .... *M. reukaufii*: p. 265
   b 2-Ketogluconate not assimilated .... *M. gruessii*: p. 260

6(4). a Glucose fermented; galactose and glucitol assimilated .... *M. zobellii*: p. 266
   b Glucose not fermented; galactose and glucitol not assimilated .... *M. krissii*: p. 262

7(4). a Glucose fermented; growth on 50% glucose agar → 8
   b Glucose not fermented; no growth on 50% glucose agar .... *M. australis*: p. 258

8(7). a Strong growth at 37°C .... *M. agaves*: p. 257
   b No growth at 37°C .... *M. hawaiiensis*: p. 261

Table 30
Key characters of species and varieties in the genus *Metschnikowia*

Species	G37°C[a]	FermG[a]	Assimilation[a]						Ploidy[b]	Spores[c]	Habitat	Mol% G+C
			Gal	D-Gluc	α-MG	2-KG	Treh	Glcn				
Metschnikowia agaves	+	+	+	+	–	v	+	w	1N	2	terrestrial	?
M. australis	–	–	+	+	–	–	+	–	1N	2	marine	47.0
M. bicuspidata var. *bicuspidata*	–	+	+	+	–	+	+	+	2N	2	aquatic	48.0
M. bicuspidata var. *californica*	–	+	+	+	+	+	+	+	2N	2	marine	47.6
M. bicuspidata var. *chathamia*	–	+	+	+	+	–	+	–	2N	2	aquatic	47.5
M. gruessii	–	l	v	–	–	–	–	v	2N	2	terrestrial	39.2
M. hawaiiensis	–	+	+	+	–	+	+	l	1N	2	terrestrial	46.7
M. krissii	–	–	–	–	+	–	+	–	2N	1	marine	45.4
M. lunata	–	+	+	+	w/–	+	+	+	2N	1 or 2	terrestrial	44.2
M. pulcherrima	v	+	+	+	+	+	+	+	2N	2	terrestrial	45.6
M. reukaufii	v	+	v	+	v	+	+	v	2N	2	terrestrial	41.3
M. zobellii	–	+	+	+	+	–	+	–	2N	1	marine	47.5

[a] Abbreviations: G37°C, growth at 37°C; FermG, fermentation of glucose; Gal, D-galactose; D-Gluc, D-glucitol; α-MG, α-methyl-D-glucoside; 2-KG, 2-keto-D-glucoside; Treh, trehalose; Glcn, gluconate.
[b] Ploidy of vegetative cells.
[c] Number of spores per ascus.

Systematic discussion of the species

39.1. *Metschnikowia agaves*[1] Lachance (1993)

Growth in glucose (2%) yeast extract (0.5%) broth: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, occur singly, in parent–bud pairs, or occasionally in short chains and measure (2–4)×(3–7) µm. A sediment and a diffuse but thick ring are formed after one month.

Growth on 5% malt agar: After 2 weeks at 17°C, colonies are small, low convex, glabrous, smooth, white and butyrous to chalky.

Dalmau plate cultures on corn meal agar: After 2 weeks, no pseudohyphae or true hyphae are formed.

Formation of ascospores: After 1 day on Yeast Carbon Base agar (without added nitrogen source), mixed strains of complementary mating types give rise to long (several cell lengths) conjugation tubes, or elongated cells. Conjugated pairs and zygotes are also present. After 3 days at 25°C, mature asci are present, pleiomorphic in shape but usually quasi-cylindrical and conjugated. Two acicular ascospores, 15–20 µm in length, are formed in one of the two conjugated parent cells forming the ascus (Fig. 112). In some cases the spores stretch the ascus into a single tubular shape. Ascospores are not liberated at maturity.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	l
Maltose	–	Cellobiose	l

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	s
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	w
Soluble starch	–	DL-Lactate	–
D-Xylose	ws	Succinate	s
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	w
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	w/–	Starch synthesis	–
5-Keto-D-gluconate	–	Urease	–
Cadaverine	+	Gelatin hydrolysis	–
L-Lysine	+	Casein hydrolysis	–
Ethylamine	+	Cycloheximide 10 µg/ml	–
50% Glucose	s	Acid production	–
10% NaCl/5% glucose	+	Growth at 37°C	+

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: Both type and isotype strains were isolated from basal rots of leaves of blue agave plants (*Agave tequilana* var. *azul*), Jalisco, Mexico.

[1] The original spelling of the species name as *agaveae* has been treated as an orthographic error.

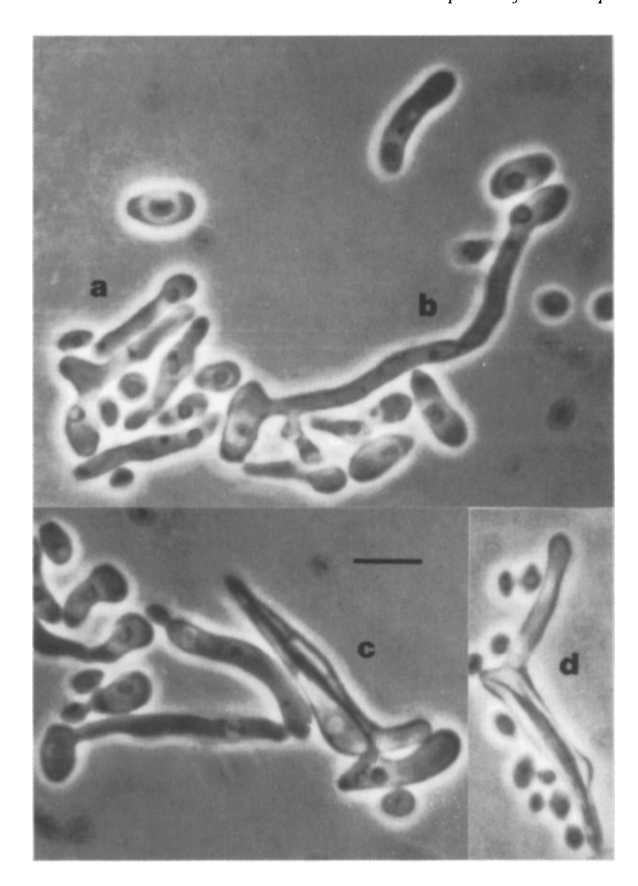


Fig. 112. Phase contrast micrographs of *M. agaves*. One day after mixing compatible strains on Yeast Carbon Base agar (with no added nitrogen), cells with conjugation tubes or elongated cells (a) fuse to form zygotes (b), which after three days give rise to asci (c,d) containing two (d) acicular ascopores. Bar = 5 μm. Courtesy Lachance (1993).

Complementary mating types: CBS 7744, mating type h^+ and CBS 7745 (UWO(PS) 92-210.1), mating type h^-.

Type strain: CBS 7744 (UWO(PS) 92-207.1, designated as mating type h^+.

Comments: The characteristics of this species are from Lachance (1993) as the culture was not available for study. *Metschnikowia agaves,* like *M. hawaiiensis* and *M. australis,* occurs in nature in the form of separate haploid mating types. Lachance mixed the mating types of *M. hawaiiensis* and of *M. agaves* in all possible combinations on Yeast Carbon Base and examined the mixtures after 3 and 7 days. He observed intraspecific crosses in both species, resulting in abundant asci and spores, whereas the four interspecific crosses showed no sign of mating activity. He considered this as evidence that *M. agaves* represented a distinct species. *M. agaves* grows well at 37°C in contrast to other haploid *Metschnikowia* species and is unable to assimilate glycerol.

39.2. *Metschnikowia australis* (Fell & I.L. Hunter) Mendonça-Hagler, Phaff & Tredick (1985)

Synonym:

Metschnikowia bicuspidata var. *australis* Fell & I.L. Hunter (1968)

Growth in 5% malt extract: After 7 days at 18°C, cells are spheroidal to ovoidal, (3–5) × (6–8) μm, single, in pairs or small groups. After 2 weeks there is a sediment and a light ring, but no pellicle.

Growth on 10% malt agar: Cell morphology is similar to that in malt extract. After 2 weeks the streak culture is semi-dull, smooth, white, texture soft, the cross-section is flat and the periphery entire.

Dalmau plate culture on corn meal agar: At 18°C pseudomycelium formation is absent or spotty. If present, its development is rudimentary.

Formation of ascospores: Following the conjugation of two haploid cells of complementary mating types, a large clavate ascus develops, containing two acicular spores. Asci and spores vary in length from about 25 to 40 μm. Asci do not lyse or discharge the spores at maturity.

Sporulation occurs on dilute (2%) malt agar at 12°C after 5–10 days.

Fermentation: absent.

Assimilation:

In YNB media + 2 % NaCl at 18°C.

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	+/w	Methanol	–
L-Sorbose	w/–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	l/w	Salicin	l/w
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	l/w	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Lipase	–
5-Keto-D-gluconate	–	Gelatin hydrolysis	–
Cadaverine	w	Casein hydrolysis	–
L-Lysine	+	Cycloheximide 10 μg/ml	+
Ethylamine	+	Cycloheximide 100 μg/ml	–
50% Glucose	–	Acid production	–
10% NaCl/5% glucose	+	Growth at 25°C	+
Starch synthesis	–	Growth at 27°C	–
Urease	–		

Co-Q: 9 (Y. Yamada, personal communication, 1990).

Mol% G + C: 47.0, CBS 5847, mating type *a* (BD: Mendonça-Hagler et al. 1985).

Origin of the strains studied: CBS 5847, CBS 5848, UCD-FST 68-48, 68-49, 68-50 and 68-51. All strains were isolated from antarctic seawater and obtained from J.W. Fell.

Complementary mating types: CBS 5847 mating

type *a*, and CBS 5848 mating type α, both isolated as haploid cells.

Type strain: CBS 5847 (NRRL Y-7013), designated as the holotype.

Comments: Mendonça-Hagler et al. (1985) investigated the DNA relatedness among aquatic members of the genus *Metschnikowia*. *M. bicuspidata* var. *australis* DNA showed 37–51% relative binding with the DNAs of *M. bicuspidata* var. *bicuspidata* and of its varieties *chathamia* and *californica* (Fell and Hunter 1968, Pitt and Miller 1970a). On this basis, as well as reduced intervarietal fertility (Pitt and Miller 1970b) and unique habitat in antarctic seawater, Mendonça-Hagler et al. (1985) proposed raising the variety *australis* to the rank of species, which we support.

39.3. *Metschnikowia bicuspidata* (Metschnikoff) Kamienski (1899)

This species has three varieties:

Metschnikowia bicuspidata (Metschnikoff) Kamienski var. *bicuspidata* (1969)

Synonyms:

Monospora bicuspidata Metschnikoff (1884)
Monosporella bicuspidata (Metschnikoff) Keilin (1920)
Metschnikowiella bicuspidata (Metschnikoff) Kudryavtsev (1960)
Metschnikowia kamienskii Spencer, Phaff & Gardner (1964)
Metschnikowia wickerhamii Codreanu & Codreanu-Balcescu (1981)

Metschnikowia bicuspidata var. californica Pitt & M.W. Miller (1970a)

Metschnikowia bicuspidata var. chathamia Fell & Pitt (1969)

Growth in 5% malt extract: After 3 days at 25°C, vegetative cells are mostly ellipsoidal to cylindroidal (3–8)×(3–13) μm, in some strains globose, 5–8 μm, or elongate, 23–35 μm, in length. Reproduction is by multilateral budding, cells occur singly or are catenulate. After one month, large refractile globose cells, 7–10 μm, containing a lipid globule, may be present. A ring and abundant sediment are formed, a pellicle is absent.

Growth on 10% malt agar: After one month at 25°C, the growth is cream-colored, the surface is smooth, or in occasional strains rugose, the edge entire or crenulate.

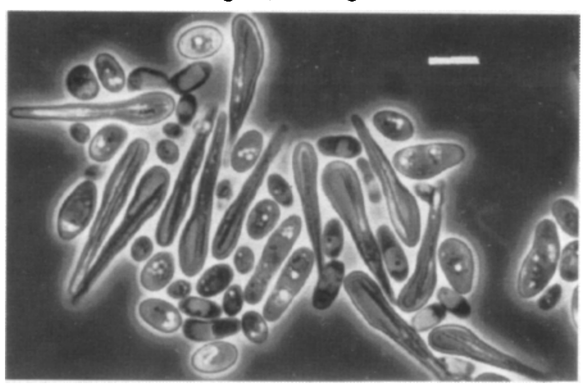


Fig. 113. *M. bicuspidata* var. *bicuspidata*, CBS 5575. Asci formed on V8 agar after 8 days at 25°C. Bar = 10 μm.

Dalmau plate culture on corn meal agar: Rudimentary pseudomycelium is formed, usually profusely.

Formation of ascospores: Asci arise from vegetative cells and are generally clavate, although less regular shapes are also formed, measuring (3–7)×(15–45) μm, sometimes to 60 μm, containing two, often appearing as one, acicular ascospores (0.7–1.1)×(15–50) μm (Fig. 113). Asci have been observed to discharge the spores in some strains through the tip.

Sporulation occurs after 7–20 days on V8 agar or on diluted (1:9) V8 agar at 14–25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+/w
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	v	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	–
5-Keto-D-gluconate	–	Gelatin hydrolysis	–
Cadaverine	+	Casein hydrolysis	–
L-Lysine	+	Cycloheximide 1 μg/ml	w/–
Ethylamine	+	Acid production	–
50% Glucose	–	Growth at 30°C	v
12.5% NaCl/5% glucose	+	Growth at 37°C	–
Starch synthesis	–		

Co-Q: 9 (Yamada et al. 1977).
Mol% G + C: 48.0, CBS 5575 (BD: Mendonça-Hagler et al. 1985).

Supplementary description of *M. bicuspidata* var. *californica*: The variety *californica* assimilates α-methyl-D-glucoside well, shows strong growth in the presence of 1 μg/ml cycloheximide and weak growth with 10 μg/ml. These differences allow it to be separated from the variety *bicuspidata* using standard growth tests.

Mol% G + C: 47.6, CBS 6010 (BD: Mendonça-Hagler et al. 1985).

DNA complementarity between the variety *californica* and the type strain of the variety *bicuspidata* was reported to be 86% (Mendonça-Hagler et al. 1985). The habitat of the two varieties differs. All strains of the variety *californica* came from sea water or kelp off the coast

of southern and central California, while most strains of the variety *bicuspidata* originated in salt lakes, often associated with diseased brine shrimp, and sometimes in fresh water lakes, possibly associated with *Daphnia magna* or with trematode sporocysts.

Supplementary description of *M. bicuspidata* var. *chathamia*: The variety *chathamia* assimilates α-methyl-D-glucoside well, but does not assimilate D-gluconate and 2-keto-D-gluconate. These differences allow it to be separated from the variety *bicuspidata* using standard growth tests. In addition, some strains grow weakly or latently on ribitol. Growth in the presence of 1 μg/ml cycloheximide is good, but absent with 10 μg/ml. Maximum growth temperature is 30°C. DNA complementarity between the variety *chathamii* and the type strain of the variety *bicuspidata* was reported to be 81%.

Mol% G+C: 47.5 (BD: Mendonça-Hagler et al. 1985).

Origin of the strains belonging to the variety *bicuspidata*: Sporocysts of *Diplostomum flexicaudum* (5); brine shrimp (*Artemia salina*) (13); *Castolia odorata* from a lake in Wisconsin (1).

Complementary mating types: CBS 5902 (*a*) and CBS 5903 (*α*).

Type strain: CBS 5575, selected as neotype by L.J. Wickerham, who first isolated it in culture from sporocysts of the parasitic strigeid trematode *Diplostomum flexicaudum* inhabiting the digestive gland of the snail *Stagnicola emarginata angulata* in a fresh water lake in Michigan.

Origin of the strains belonging to the variety *californica*: Sea water or kelp off the coast of California from San Diego to Point Reyes in central California (23).

Type strain: CBS 6010 (UCD-FST 67-100) isolated by Phaff from Pacific ocean water near Point Reyes, California.

Origin of the strains belonging to the variety *chathamia*: Fresh water ponds on Chatham Island, New Zealand (3).

Type strain: CBS 5980 (IMS 23-414, UCD-FST 67-2) isolated by Fell from a fresh water pond near the ocean on Chatham Island.

Comments: Retaining the three varieties of *M. bicuspidata* (Table 30) was based on differences in metabolic profiles, biogeography and habitat. The varieties show interfertility (Pitt and Miller 1970a) and DNA relatedness values ranging from 81 to 86% compared with the variety *bicuspidata* (Mendonça-Hagler et al. 1985). The former variety *australis* (Fell and Hunter 1968) was elevated to the rank of species by Mendonça-Hagler et al. (1985).

Strains of the three varieties comprising the species *M. bicuspidata* are morphologically and physiologically quite similar, and related by their fresh water or marine habitat, ascus shape, number of ascospores and interfertility between mating types. The variety *bicuspidata* is generally homothallic although heterothallic haploids have been obtained (Wickerham 1964a), apparently by a parasexual mechanism as described by Pitt and Miller (1970b). These haploids are capable of hybridizing with the other heterothallic varieties and producing sparsely sporulating hybrids.

Some strains of the variety *bicuspidata,* i.e., those designated as *M. kamienskii,* in general had slightly lower maximum temperatures for growth (27–28°C) than the strains obtained from sporocysts which generally grew at 30–34°C.

The nine strains of the variety *bicuspidata* which we isolated from diseased *Artemia salina* taken from salt ponds (ca. 10% NaCl) in southern California (Lachance et al. 1976), initially did not grow in the usual assimilation media unless they were supplemented with 2% NaCl.

Asci of these strains have been observed to discharge their spores from the tip of the peduncle, occasionally quite forcefully. The ecological implication and the possibility of active mechanical predation for the variety *bicuspidata* have been discussed by Lachance et al. (1976), who misidentified these strains as *M. bicuspidata* var. *australis.*

39.4. *Metschnikowia gruessii* Giménez-Jurado (1992)

Synonyms:

Anthomyces reukaufii Grüss (1918)
Nectaromyces reukaufii (Grüss) H. Sydow & P. Sydow (1918)
Nectaromyces cruceatus Schoellhorn (1919)

Growth in glucose (2%)–yeast extract (0.5%)–peptone (1%) broth: After 3 days at 25°C, the cells are variable in shape and size, ovoidal, ellipsoidal, sometimes cylindrical and measure (2.5–6.3)×(8.8–27.5) μm. Cells are usually in pairs or in groups of four giving rise to characteristic "airplane" or trident configurations. After one week, refractile, ovoidal chlamydospores, (5.0–12.5)×(10.0–15.0) μm, with one or more lipid globules are present. No ring or pellicle is formed.

Growth on glucose (2%)–yeast extract (0.5%)–peptone (1%) agar: After one week at 25°C, most strains form colonies which are cream-colored, smooth, often papillate and glistening. Colony shape is slightly raised and convex with an entire margin. Texture is butyrous.

Dalmau plate cultures on corn meal agar: After 7 days at 25°C, growth under the coverslip consists of short chains of stalagmoid cells with ovoid blastoconidia. Chlamydospores and "airplane" configurations are also present.

Formation of ascospores: Asci may originate from cells and from chlamydospores which elongate at one end to a peduncle. The ellipsoidopedunculate asci measure (6.3–7.5)×(31.3–60.0) μm and contain 2 acicular to acerose ascospores that are pointed at one end, often appearing bifurcate, and are not released from the ascus at maturity.

Sporulation occurs after one week on dilute (1:14) V8 agar at 17°C.

Fermentation:

Glucose	l	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	w
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	w
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	w
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	–
5-Keto-D-gluconate	–	Gelatin hydrolysis	–
Cadaverine	+	Casein hydrolysis	–
L-Lysine	+	Cycloheximide 1 µg/ml	–
Ethylamine	+	Acid production	–
50% Glucose	–	Growth at 31°C	w
12.5% NaCl/5% glucose	+	Growth at 37°C	–
Starch synthesis	–		

Co-Q: 9 (79%), Q-8 (9.2%) (Giménez-Jurado 1992).
Mol% G+C: 39.2, CBS 7657 (T_m: Giménez-Jurado 1992).

Origin of the strain studied: Only the type strain was available to us for study and most of the characteristics given are based on the publication by Giménez-Jurado (1992).

Type strain: CBS 7657 (IGC 4382) isolated from the nectaries of *Hebe salicifolia*, Oeiras, Portugal.

Comments: Henriques et al. (1991) developed a ribosomal DNA spacer probe specific for the type strain of *M. reukaufii*. While the probe did not hybridize with other species of *Metschnikowia*, they noted that one strain identified as *M. reukaufii* also did not hybridize with the probe. DNA–DNA hybridization between *M. reukaufii* and the atypical strain revealed only 10% DNA complementarity. In addition, the G+C content of the DNA of the atypical strain was found to be approximately 4 mol% lower than that of the type strain of *M. reukaufii*, virtually precluding conspecificity (Kurtzman and Phaff 1987). Henriques et al. (1991) concluded that the non-reacting strain represented a species different from *M. reukaufii*. A detailed description of this strain as a new species of the genus *Metschnikowia*, *M. gruessii*, was subsequently published by Giménez-Jurado (1992). Besides low DNA reassociation values between the DNAs of *M. gruessii*, *M. reukaufii*, and of several additional strains, Giménez-Jurado was able to separate *M. gruessii* phenotypically by the lack of assimilation of trehalose versus strong growth by *M. reukaufii* and the formation of airplane or trident configurations in a 20% honey solution. Additionally, we found in our review that *M. gruessii* does not grow on 2-keto-D-gluconate, whereas *M. reukaufii* shows good growth on this compound.

Giménez-Jurado (1992) also noted that *M. gruessii* and *M. reukaufii* frequently occur side by side in various flower species, but that the latter is far more common. Included in Giménez-Jurado's study was an authentic strain, labeled *Nectaromyces reukaufii* (IGC 3275), that had been studied by Grüss (1918) and various later investigators, who considered it as a synonym of *Candida reukaufii* or an anamorph of *M. reukaufii*. This strain shared 97% of its base sequences with *M. gruessii*, evidencing synonymy with that species. We also have listed *Anthomyces reukaufii* as a synonym of *M. gruessii*, as this was the name originally given by Grüss to the strain that was later renamed *Nectaromyces reukaufii* by H. Sydow and P. Sydow (1918) because *Anthomyces* was a later homonym and thus illegitimate. *Nectaromyces cruciatus* is also assumed to be a synonym of *M. gruessii* because Schoellhorn (1919), who also studied the isolate of Grüss, described it as *Nectaromyces cruciatus*, apparently unaware of the other publications on this yeast cited above.

39.5. *Metschnikowia hawaiiensis* Lachance, Starmer & Phaff (1990)

Growth in 5% malt extract: After 3 days at 25°C, the haploid cells are short ovoidal to ellipsoidal, occur singly, in pairs or as cells with several buds, and measure (2–4)×(4–6) µm. A sediment is formed after 2 to 3 weeks.

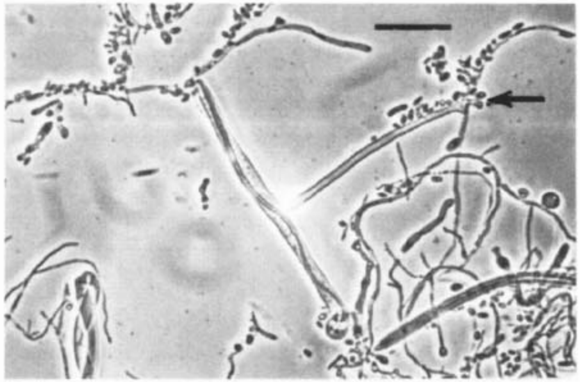
Growth on 10% malt extract agar: After 2 weeks at 17°C, colonies are large, low convex to umbonate, glabrous, smooth, white, and butyrous. Some cells develop long tubular outgrowths instead of buds.

Dalmau plate cultures on corn meal agar: Branching true hyphae with rare, phase-dark septa are formed after 2 weeks.

Formation of ascospores: As early as 6 hours after mixing of complementary mating types on 1% malt extract agar, conjugation tubes appear on some cells. After 8 hours conjugated pairs and zygotes are present, some with wide, tubular outgrowths. After 1 day, tubular, conical asci, some exceeding 200 µm in length, may be formed (Fig. 114). Most asci retain signs of the original conjugating pair of parental cells. Two large acicular ascospores (140–180 µm long) are produced per mature ascus (Fig. 115). The spores are not released from the ascus at maturity.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		



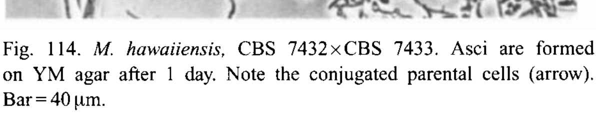


Fig. 114. *M. hawaiiensis*, CBS 7432×CBS 7433. Asci are formed on YM agar after 1 day. Note the conjugated parental cells (arrow). Bar = 40 μm.



Fig. 115. *M. hawaiiensis*, CBS 7432×CBS 7433. Pair of ascospores released from an ascus after treatment with yeast lytic enzyme (ICN Biochemicals). Bar = 40 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	w
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	ws
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	+/w
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Lipase	w
5-Keto-D-gluconate	–	Gelatin hydrolysis	–
Cadaverine	+	Casein hydrolysis	–
L-Lysine	+	Cycloheximide 1 μg/ml	+
Ethylamine	+	Cycloheximide 10 μg/ml	–
50% Glucose	+	Acid production	w/–
12.5% NaCl/5% glucose	+/s	Growth at 30°C	+
Starch synthesis	–	Growth at 37°C	–
Urease	–		

Co-Q: Not determined.

Mol% G + C: 46.6–46.7, two strains, CBS 7432 (type) and CBS 7433 (isotype) (BD: Lachance et al. 1990).

Origin of the strains studied: Morning glory flowers (*Ipomoea acuminata*) on the island of Hawaii (5); *Scaptomyza calliginosa* (7) and *Drosophila floricola* (1), both of which breed in morning glory flowers on the island of Hawaii.

Complementary mating types: CBS 7432 and CBS 7433.

Type strain: CBS 7432, isolated from a morning glory flower, island of Hawaii.

39.6. *Metschnikowia krissii* (van Uden & Castelo-Branco) van Uden (1962)

Synonym:
Metschnikowiella krissii van Uden & Castelo-Branco (1961)

Growth in 5% malt extract: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (4.5–6)×(6–11) μm; elongate cells, (4.5–6)×(11–13) μm, also occur. A thin ring may be formed.

Growth on 10% malt agar: After one month at 25°C, the streak culture is yellowish, semidull, soft, and delicately punctulate to smooth.

Dalmau plate culture on corn meal agar: A rudimentary pseudomycelium is formed.

Formation of ascospores: Asci arise from diploid vegetative cells and are generally clavate, 18–26 μm long, and contain a single, needle-shaped ascospore, which is pointed at one end and nearly as long as the respective ascus.

Asci were observed on V8 agar after 10–14 days at 16°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	w
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	–
5-Keto-D-gluconate	–	Gelatin hydrolysis	–
Cadaverine	+	Casein hydrolysis	–
L-Lysine	+	Cycloheximide 1 µg/ml	–
Ethylamine	+	Acid production	–
50% Glucose	–	Growth at 30°C	+
12.5% NaCl/5% glucose	+	Growth at 37°C	–
Starch synthesis	–		

Co-Q: 9 (Yamada et al. 1977).
Mol% G+C: 45.4, CBS 4823 (BD: Mendonça-Hagler et al. 1985).

Origin of the strains studied: Sea water off the southern California coast (4).

Type strain: CBS 4823, isolated by van Uden and Castelo-Branco (1961) from sea water off La Jolla, California.

39.7. *Metschnikowia lunata* Golubev (1977)

Synonyms:
Schizoblastosporion kobayashii Soneda & Uchida (1971)
Selenotila intestinalis Krasil'nikov (1927)
Selenozyma intestinalis (Krasil'nikov) Yarrow (von Arx et al. 1977)

Growth in 5% malt extract: After 3 days at 25°C, the cells are lunate, (2–4)×(5–8) µm, or rarely ovoidal, (2.5–5)×(3.5–8) µm, and single or in pairs (Fig. 116). A slight sediment is formed. After one month at 20°C, a sediment and ring are present. Pulcherrima cells, (8–10.5)×(8–12) µm, are present and may have two small, pointed protrusions.

Growth on 10% malt agar: After one month the streak culture is cream-colored, mat, with a smooth surface, pasty, slightly convex with an entire border. Pulcherrimin is not produced.

Dalmau plate culture on corn meal agar: A rudimentary pseudomycelium is formed.

Formation of ascospores: Asci arise from spheroidal chlamydospores, which generally contain a single, prominent lipid globule. The lipid globule is no longer present in the ascus. Asci are ellipsoidopedunculate; the ellipsoidal part measures (7–10)×(7–12) µm, whereas the peduncles are cylindrical and measure (2–4)×(13–40) µm. Asci contain one to two acicular ascospores (Fig. 117).

Asci are produced on dilute (1:9) V8 agar at 15°C after 10 days.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	w
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	w
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	w/–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	s
L-Rhamnose	–	Nitrate	–
D-Glucosamine	w	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	–
5-Keto-D-gluconate	–	Gelatin hydrolysis	–
Cadaverine	+	Casein hydrolysis	–
L-Lysine	+	Cycloheximide 1 µg/ml	+
Ethylamine	+	Cycloheximide 10 µg/ml	–
50% Glucose	w/–	Acid production	–
12.5% NaCl/5% glucose	+	Growth at 30°C	+
Starch synthesis	–	Growth at 37°C	–

Co-Q: 9 (Y. Yamada, personal communication, 1990).
Mol% G+C: 44.2, CBS 5946 (BD: Mendonça-Hagler et al. 1985).

Origin of the strains studied: CBS 5946 from a flower of *Vicia cracca* L., Russia; CBS 6798 (type of *Schizoblastosporion kobayashii*), from exudate of

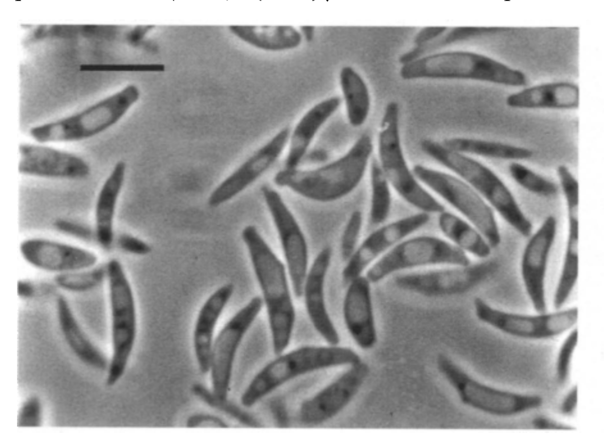


Fig. 116. *M. lunata*, CBS 5946. Lunate vegetative cells grown in malt extract for 4 days. Bar = 10 µm.

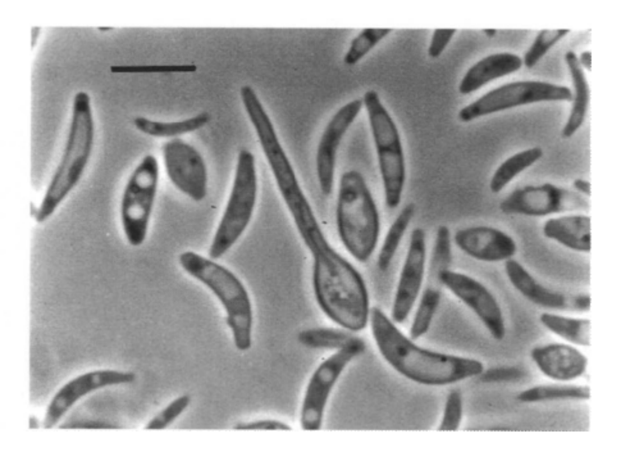


Fig. 117. *M. lunata*, CBS 5946. Ascus formed on V8 agar at 15°C. Bar = 10 µm.

Casuarina sp., Japan. This strain produced asci but no ascospores were observed.

Type strain: CBS 5946 ex flower, Russia.

39.8. *Metschnikowia pulcherrima* Pitt & M.W. Miller (1968)

Anamorph: *Candida pulcherrima* (Lindner) Windisch

Synonyms:

Torula pulcherrima Lindner (1901)
Torulopsis pulcherrima (Lindner) Saccardo (1906)
Saccharomyces pulcherrimus (Lindner) Beijerinck (1912)
Eutorula pulcherrima (Lindner) Will (1916)
Rhodotorula pulcherrima (Lindner) Harrison (1928)
Candida pulcherrima (Lindner) Windisch (1940)
Chlamydozyma pulcherrima Wickerham (1964a)
?*Torulopsis rosea* Berlese (1895)
Torula rubefaciens Grosbüsch (1915)
Monilia castellanii Re (1925)
Cryptococcus interdigitalis Pollacci & Nannizzi (1926)
Torulopsis interdigitalis (Pollacci & Nannizzi) Krasil'nikov (1954c)
Cryptococcus castellanii (Re) Castellani (1928)
Torulopsis castellanii (Re) Castellani & Jacono (1933)
Castellania castellanii (Re) Dodge (1935)
Torulopsis pulcherrima (Lindner) Saccardo var. *variabilis* Lodder (1934)
Torulopsis pulcherrima (Lindner) Saccardo var. *rubra* Castelli (1940)
Asporomyces uvae Mrak & McClung (1940)
Torulopsis dattila (Kluyver) Lodder var. *rohrbachense* von Szilvinyi & Kaulich (1948)
Torulopsis burgeffiana Benda (1962)

Growth in 5% malt extract: After 3 days at 25°C, vegetative cells are globose to ellipsoidal, (2.5–6)×(4–10) µm, usually (2.5–5)×(4–7) µm, single, reproducing by multilateral budding. Chlamydospores (pulcherrima cells) are not present. After one month at 25°C, pulcherrima cells are usually present, highly refractile, globose, (4)×(7–11) µm, or subglobose, containing a single lipid globule, occasionally more than one (Fig. 118); a thin ring and abundant sediment are present, a pellicle is absent.

Growth on 10% malt agar: After one month at 25°C, growth is cream-colored, or if pulcherrimin pigment is produced, reddish-brown (sometimes in sectors). The surface is smooth and glistening, in some strains sparsely papillate, edge entire. Pulcherrimin pigment usually diffuses into the medium.

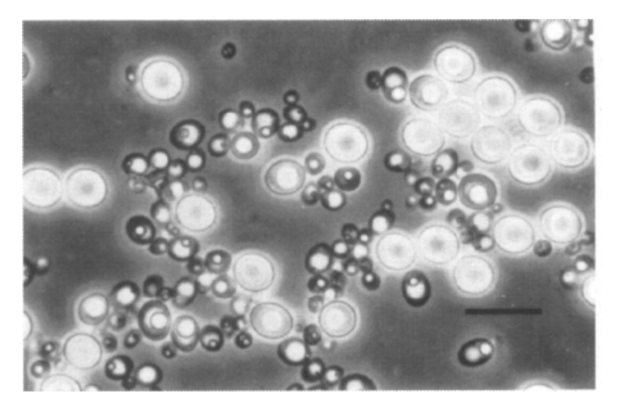


Fig. 118. *M. pulcherrima,* CBS 5833. Vegetative and pulcherrima cells on malt agar after 6 days. Bar = 10 µm.

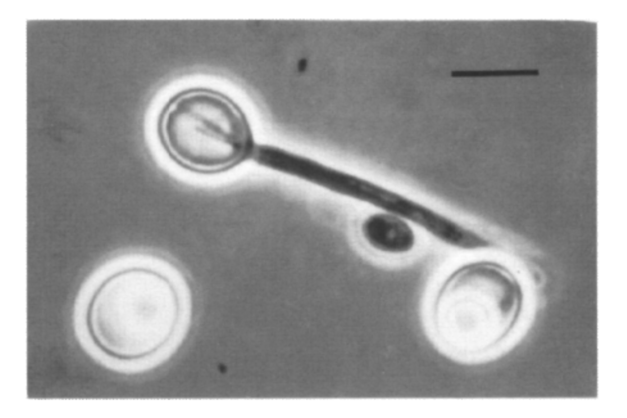


Fig. 119. *M. pulcherrima,* CBS 5833. Ascus with distinctly separated ascospores formed on dilute, filtered V8 agar incubated for 2 weeks at 15°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: Aerobically, pseudomycelium is not produced; anaerobically, rudimentary pseudomycelium is formed, often profusely.

Formation of ascospores: Asci arise from spheroidal chlamydospores, with one or more prominent lipid globules (Fig. 118). The lipid globules disappear during ascus development. Asci are sphaeropedunculate, (4–11)×(15–55) µm, usually (6–8)×(20–42) µm, peduncles are cylindrical, (1.0–2.5)×(10–45) µm, usually (1.5–2)×(15–30) µm, containing two, sometimes one, acicular to filiform ascospores, (0.4–1.5)×(9–27) µm (Fig. 119). In some strains, asci were observed to lyse at the peduncle extremity.

Asci are formed on dilute, filtered V8 juice agar, and other dilute media (1:9; 1:29), after incubation for 5–21 days at 12–21°C (Pitt and Miller 1968).

Fermentation:

Glucose	+	Lactose	–
Galactose	w/–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w/–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	w/–	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	−
5-Keto-D-gluconate	−	Gelatin hydrolysis	−
Cadaverine	+	Casein hydrolysis	−
L-Lysine	+	Cycloheximide 10 μg/ml	+
Ethylamine	+	Cycloheximide 100 μg/ml	−
50% Glucose	+	Acid production	−
12.5% NaCl/5% glucose	w	Growth at 30°C	+
10% NaCl/5% glucose	+	Growth at 37°C	v
Starch synthesis	−		

Co-Q: 9 (Yamada and Kondo 1972b).
Mol% G+C: 45.6, CBS 5833 (BD: Mendonça-Hagler et al. 1985).

Origin of the strains studied: Flowers from N.W. Canada (9); flowers and spoiled fruit in California (11); *Drosophila* spp. (4); from exudates of buckeye (*Aesculus*), birch (*Betula*), and willow (*Salix*) spp. in Japan and the U.S. Pacific Northwest (4).

Type strain: CBS 5833, isolated by Mrak and McClung (1940) from grapes.

Comments: As is evident from the number of synonyms of *M. pulcherrima,* a number of yeasts or yeastlike isolates were not recognized initially as members of that species or its anamorph. For example, Castellani (1925) isolated several yeast strains from diseased tissues of human patients which he referred to as *Monilia* sp. or *Cryptococcus* sp. Re (1925) subsequently described these isolates as *Monilia castellanii* or *Cryptococcus castellanii* to provide tentative binomials for the isolates. Castellani (1928) referred to the isolates as *Cryptococcus castellanii* but he and Jacono (1933) changed the name to *Torulopsis castellanii.* Finally, Langeron and Guerra (1941) recognized that these strains were representatives of *Torulopsis pulcherrima,* because they produced a characteristic maroon pigment on media supplemented with ferric ions and produced large lipid-containing chlamydospores.

Langeron and Guerra (1941) also reidentified a species named *Cryptococcus interdigitalis* by Pollacci and Nannizzi (1926) as a strain of *Torulopsis pulcherrima.* Lodder (1934) had previously considered *Cryptococcus interdigitalis* as a variety of *T. pulcherrima.*

39.9. *Metschnikowia reukaufii* Pitt & M.W. Miller (1968)

Anamorph: *Candida reukaufii* (Grüss) Diddens & Lodder
Synonyms:
Candida reukaufii (Grüss) Diddens & Lodder (1942)
Chlamydozyma reukaufii Wickerham (1964a)
Chlamydozyma zygota Wickerham (1964a)
Metschnikowia zygota Fell & I.L. Hunter (1968)
?*Candida rancensis* C. Ramírez & A. González (1984h)

Growth in 5% malt extract: After 3 days at 25°C, vegetative cells are ellipsoidal to cylindroidal, (2–6)×(4–18) μm, usually (2–4)×(6–12) μm, single or catenulate, reproducing by multilateral budding. In some strains longer cells occur, up to 35 μm. Chlamydospores are not present. After one month at 25°C, somewhat elongate chlamydospores are usually present, (4–10)×(8–30) μm, but usually (8–10)×(10–20) μm. They are highly refractile, and contain several to numerous lipid globules (Fig. 120); a thin ring and abundant sediment are present; a pellicle is absent.

Growth on 10% malt agar: After one month at 25°C, growth is cream-colored, the surface is smooth and glistening, in some strains sparsely papillate, and the edge is entire to crenulate.

Dalmau plate culture on corn meal agar: Aerobically, pseudomycelium is not produced; anaerobically, rudimentary pseudomycelium is profusely formed.

Formation of ascospores: Asci arise from somewhat elongated chlamydospores that contain several to numerous lipid globules. Asci are ellipsoidopedunculate to clavate, (5–9)×(20–43) μm, usually (5–8)×(30–37) μm, containing two (rarely one) acicular to acerose ascospores, (0.7–1.1)×(7–30) μm (Fig. 121). Asci do not lyse at maturity.

Asci develop on dilute (1:2 to 1:29), filtered V8 juice agar, and other dilute media after 7–21 days at 12–21°C (Pitt and Miller 1968).

Fermentation:

Glucose	+	Lactose	−
Galactose	w/−	Raffinose	−
Sucrose	−	Trehalose	−
Maltose	−		

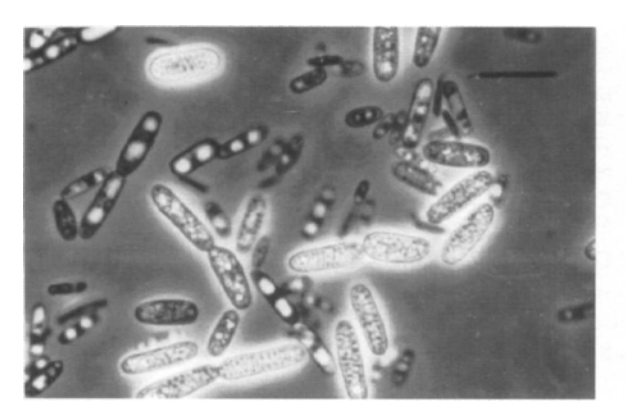


Fig. 120. *M. reukaufii*, CBS 5834. Vegetative and reukaufii cells formed in malt extract. Bar = 20 μm.

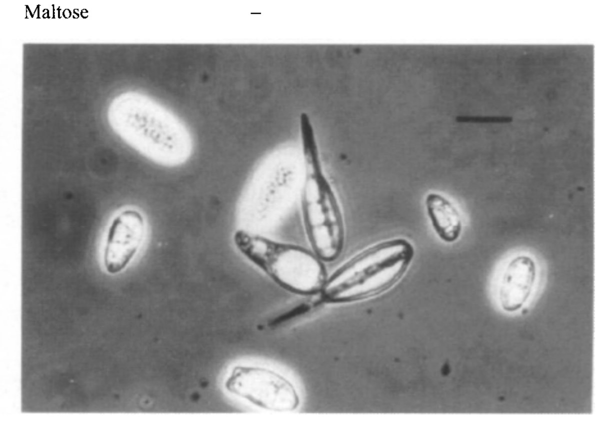


Fig. 121. *M. reukaufii*, CBS 5834. Ellipsoidopedunculate asci and reukaufii cells on dilute V8 agar after 2 weeks at 18°C. Bar = 10 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	w
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	w
L-Rhamnose	–	Nitrate	–
D-Glucosamine	w/–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	–
5-Keto-D-gluconate	–	Gelatin hydrolysis	–
Cadaverine	+	Casein hydrolysis	+
L-Lysine	+	Cycloheximide 1 µg/ml	+
Ethylamine	+	Cycloheximide 10 µg/ml	–
50% Glucose	+	Acid production	–
10% NaCl/5% glucose	+	Growth at 30°C	+
12.5% NaCl/5% glucose	w	Growth at 37°C	–
Starch synthesis	–		

Co-Q: 9 (Y. Yamada, personal communication, 1990).
Mol% G+C: 41.3, CBS 5834 (BD: Mendonça-Hagler et al. 1985).

Origin of the strains studied: All strains were obtained from flowers over a wide geographic area, near Fort Smith, North West Territory, Canada (17); Saskatoon, Saskatchewan, Canada (5); Davis, Calif. (1); Japan (1); State of Washington, U.S.A. (1).

Type strain: CBS 5834 (UCD-FST 62-311) diploid, isolated by J.F.T. Spencer from fireweed flowers (*Epilobium angustifolium* at Fort Smith, N.W.T., Canada.

39.10. *Metschnikowia zobellii* (van Uden & Castelo-Branco) van Uden (1962)

Synonyms:
Metschnikowiella zobellii van Uden & Castelo-Branco (1961)
Metschnikowia bicuspidata (Metschnikoff) Kamienski var. *zobellii* Fell & I.L. Hunter (1968)

Growth in 5% malt extract: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (6–11)×(7–11) µm. A pellicle and a ring may be formed.

Growth on 10% malt agar: After one month at 25°C, the streak culture is yellowish or grayish, semidull to dull, soft, delicately punctulate to smooth.

Dalmau plate culture on corn meal agar: A rudimentary pseudomycelium is formed.

Formation of ascospores: Asci arise from vegetative cells and are generally clavate, 18–24 µm long, and contain a single, needle-shaped ascospore, pointed at one end and nearly as long as the respective ascus (Fig. 122).

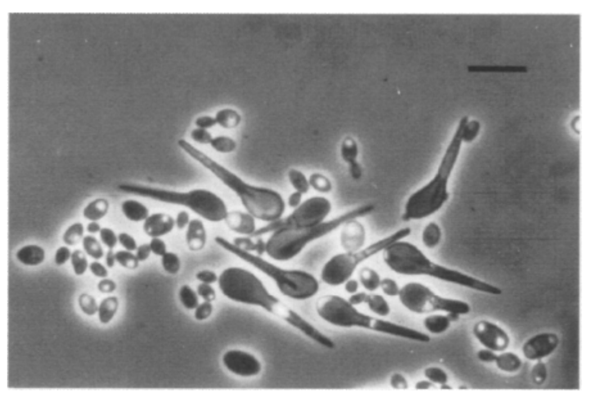


Fig. 122. *M. zobellii*, CBS 4821. Asci and vegetative cells on V8 agar after 2 weeks at 18°C. Bar = 10 µm.

Asci were observed on V8 agar after 6–20 days at 18°C.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	w	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l/w	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	–
5-Keto-D-gluconate	–	Lipase	–
Cadaverine	+	Gelatin hydrolysis	–
L-Lysine	+	Casein hydrolysis	–
Ethylamine	+	Cycloheximide 100 µg/ml	+
50% Glucose	w	Acid production	–
12.5% NaCl/5% glucose	+	Growth at 30°C	+
Starch synthesis	–	Growth at 35°C	–

Co-Q: 9 (Yamada et al. 1977).
Mol% G+C: 47.5, CBS 4821 (BD: Mendonça-Hagler et al. 1985).

Origin of the strains studied: Sea water and kelp off the southern California coast (3); gut content of fish, La Jolla, southern California (1); sea water of the Clyde estuary, Scotland (4).

Type strain: CBS 4821, isolated by van Uden and Castelo-Branco (1961) from sea water off La Jolla, California.

Comments on the genus

The 10 species in the genus *Metschnikowia* are characterized by the formation of long needle-shaped or acicular ascospores without appendages. The asci are much larger than the vegetative cells. Six of the species have a terrestrial habitat and are usually found in the corolla of flowers or in decaying fruit or plant tissue; they are transmitted to new niches by insects, such as bees and drosophilids. The other four species (including two varieties) have aquatic habitats (marine, salt lakes or fresh water) and are usually associated with crustacean species and sometimes with fish or kelp. They may be pathogenic to certain crustaceans, e.g., brine shrimp (*Artemia*) and fresh water shrimp (*Daphnia*) (Lachance et al. 1976). Four of the terrestrial species form chlamydospores that are precursors of asci. The other two terrestrial species and the aquatic species lack chlamydospores and form asci from diploid vegetative cells or from zygotes of haploid complementary mating types (Table 30).

Characteristics differentiating the species include fermentation of glucose, assimilation of various carbon compounds (Table 30), maximum temperature for growth, growth on media with high osmotic pressure, and ploidy of the vegetative cells. The production of pulcherrimin by *M. pulcherrima* is variable and therefore not always a reliable diagnostic character. Mendonça-Hagler et al. (1993) estimated phylogenetic relationships among type strains of the eight *Metschnikowia* species known at the time of their study. The extent of divergence of partial sequences of the large and small subunits of rRNA suggested that the aquatic and terrestrial species form two clusters within the genus. Among the terrestrial species, *M. lunata* and *M. hawaiiensis* were well separated from the others, with the latter so divergent that it might even be placed in a new genus. This study did not include the two recently described terrestrial species, *M. agaves* and *M. gruessii.*

Codreanu and Codreanu-Balcescu (1981) reported isolating *Metschnikowia* strains producing monosporic asci from infected *Artemia* and *Daphnia* specimens in Rumania. They proposed that these strains represented the original isolates made by Kamienski (1899) from *Artemia salina* and from *Daphnia magna* by Metschnikoff (1884), and that Wickerham's (1964a) neotype of *M. bicuspidata,* which formed two spores per ascus, should be rejected. Unfortunately, as far as we are aware, no strains isolated by the Rumanian team have been preserved or tested physiologically. Although their electronmicrographs clearly show that the asci contain single acicular spores, it is entirely possible that the number of spores per ascus varies from one to two, as is common in many other yeast genera. The present authors have therefore retained Wickerham's two-spored isolate of *M. bicuspidata* from a strigeid trematode as the neotype of *M. bicuspidata.*

40. *Nadsonia* Sydow

M.W. Miller and H.J. Phaff

Diagnosis of the genus

Cells are lemon-shaped, ovoidal, or elongate. Vegetative reproduction by bud-fission at both poles; this process involves the formation of a bud-like structure on a very wide neck. The bud is separated by the formation of a cross wall, followed by fission. Pseudomycelium is not formed; a few chains of elongate cells may be present.

Two mechanisms of ascospore formation are known. (1) After a heterogamic conjugation between the parent cell and a bud, the contents of the zygote move into another bud formed at the opposite end of the parent cell. This second bud is then delimited by a septum and becomes the ascus. (2) After a heterogamic conjugation between the parent cell and a bud, the bud is normally delimited by a septum and the parent cell becomes the ascus. One or more, rarely two, spherical, brownish, spiny- to warty-walled spores are formed. Spores contain a prominent lipid globule.

Fermentation of glucose is present or absent. Nitrate is not utilized. No growth at 30°C or above. On malt extract a pellicle is usually formed. Diazonium blue B reaction is negative.

Type species

Nadsonia fulvescens (Nadson & Konokotina) Sydow

Species accepted

1. *Nadsonia commutata* Golubev (1973)
2. *Nadsonia fulvescens* (Nadson & Konokotina) Sydow (1912)
   a. *Nadsonia fulvescens* (Nadson & Konokotina) Sydow var. *fulvescens* (1989)
   b. *Nadsonia fulvescens* var. *elongata* (Konokotina) Golubev, M.Th. Smith, Poot & Kock (1989)

Key to species

1.	a Glucose fermented; growth in presence of 1 μg/ml cycloheximide positive	→ 2	
	b Glucose not fermented; absence of growth in presence of 1 μg/ml cycloheximide	*N. commutata*:	p. 268
2(1).	a Maltose assimilated	*N. fulvescens* var. *fulvescens*:	p. 269
	b Maltose not assimilated	*N. fulvescens* var. *elongata*:	p. 269

Systematic discussion of the species

40.1. *Nadsonia commutata* Golubev (1973)

Growth in 5% malt extract: After 3 days at 15°C, cells are ovoidal to apiculate, (4.2–7.7)×(5.6–11.2) μm, single, in pairs or in short chains; budding is bipolar on a broad base (Fig. 123). After one month a ring and sediment are formed, sometimes a pellicle.

Growth on 10% malt agar: Cell shapes and dimensions similar to those observed in malt extract. After one month at 18°C, the streak culture is cream-colored (brownish in sporulating cultures), smooth, semiglossy, texture is pasty and the border is entire to lobate.

Dalmau plate culture on potato agar: Pseudomycelium is not formed.

Formation of ascospores: Asci are formed by mechanism (2), following lysis of the wall separating a parent cell and a polar bud; the nucleus and other contents of the bud pass into the parent cell where karyogamy and meiosis take place. A new septum is produced separating the zygote from the bud. The parent cell, which has become the ascus, normally contains a single or rarely two spheroidal, spiny to warty, brownish spores with a prominent lipid globule (Fig. 124).

Sporulation occurs on YM or malt agar at 18°C in 7 days.

Fermentation: absent.

Assimilation (at 20°C):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–



Fig. 123. *N. commutata*, CBS 6640. Cells grown in YM broth, for 7 days. Bar = 10 μm.

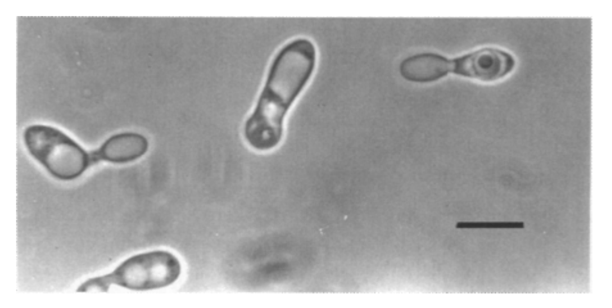


Fig. 124. *N. commutata*, CBS 6640. Ascus formed on YM agar after 7 days, 18°C. Bar = 10 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	–
5-Keto-D-gluconate	–	Lipase	–
Cadaverine	–	Gelatin hydrolysis	–
L-Lysine	–	Casein hydrolysis	–
Ethylamine	–	Cycloheximide 1 μg/ml	–
50% Glucose	–	Acid production	–
5% NaCl/5% glucose	–	Growth at 22°C	+
Starch synthesis	–	Growth at 25°C	–

Co-Q: 6 (Yamada et al. 1992c).
Mol% G + C: 40.0, CBS 6640 (BD: H.J. Phaff, unpublished).

Origin of the strains studied: CBS 6640 from field soil, East Falkland Island (South Atlantic ocean) (Golubev 1973); VKM Y-2610 from soil, Carpathian Mts., Eastern Europe.

Type strain: CBS 6640 (VKM Y-1573).

40.2. *Nadsonia fulvescens* (Nadson & Konokotina) Sydow (1912)

This species has two varieties:

***Nadsonia fulvescens* (Nadson & Konokotina) Sydow var. *fulvescens* (1989)**

Synonym:
Guilliermondia fulvescens Nadson & Konokotina (1911)

***Nadsonia fulvescens* var. *elongata* (Konokotina) Golubev, M.Th. Smith, Poot & Kock (1989)**

Synonyms:
Guilliermondia elongata Konokotina (1913)
Nadsonia richteri Kostka (1927)
Saccharomycodes sinensis Yue (1977)

Growth in 5% malt extract: After three days at room temperature, the cells are ovoidal to elongate or lemon-shaped, (5–8) × (8–16) μm, and occasionally longer, single, in pairs, or in short chains. Budding is bipolar on a broad base (Fig. 125). A septum forms across the constriction connecting bud and parent cell, and the two are separated by fission. A thin, smooth, dry creeping pellicle is present. After one month a thin pellicle and a sediment are present.

Growth on 10% malt agar: After three days at room temperature, the cells are similar to those in malt extract. After one month at 18°C, the streak culture is grayish to cream-colored, nearly smooth, pasty, semidull, raised, border entire or lobulate.

Dalmau plate culture on potato agar: Pseudomycelium absent or rudimentary; in the last case short chains of undifferentiated elongate cells are formed.

Formation of ascospores: Sporulation, although not observed in the variety *fulvescens*, takes place according to the original description as described for the genus under the Diagnosis. When an ascus of the variety *elongata* is mature it normally separates from the two empty cells of a triad by fission (Fig. 126).

Sporulation by the variety *elongata* was observed on 10% malt agar after 4 days at 18°C.

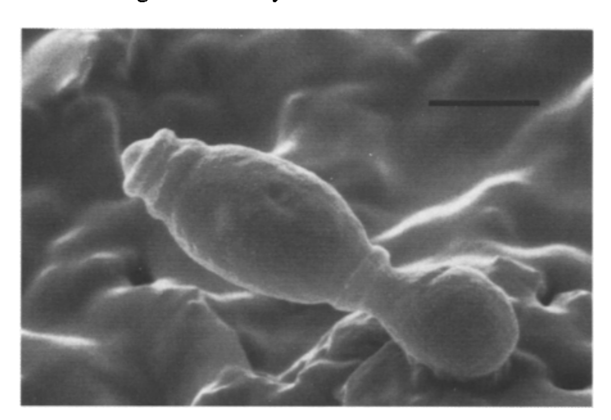


Fig. 125. *N. fulvescens* var. *elongata*, UCD-FST 67-485. Scanning electron micrograph of typical bipolar budding cell. Bar = 5 μm.

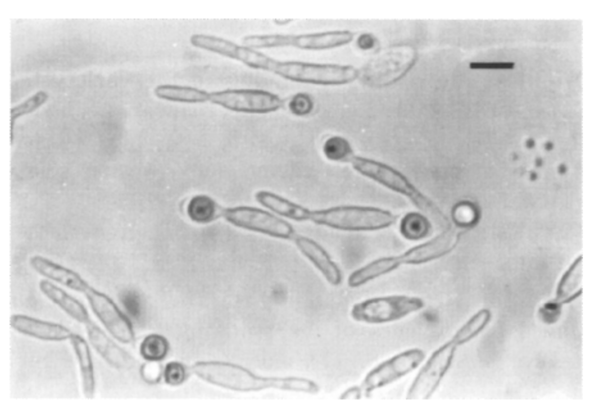


Fig. 126. *N. fulvescens* var. *elongata*, UCD-FST C98. Ascus formed by typical triad formation on malt agar after 4 days. Bar = 10 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	w		

Assimilation (at 20°C):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	w/–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Lipase	–
5-Keto-D-gluconate	–	Gelatin hydrolysis	–
Cadaverine	+	Casein hydrolysis	–
L-Lysine	+	Cycloheximide 1 μg/ml	+
Ethylamine	v	Cycloheximide 10 μg/ml	–
50% Glucose	–	Acid production	–
5% NaCl/5% glucose	–	Growth at 25°C	+
Starch synthesis	–	Growth at 30°C	–
Urease	–		

Co-Q: 6 (Yamada et al. 1976a).
Mol% G+C: 41.5, CBS 2596 (BD: H.J. Phaff, unpublished).

Supplementary description of *N. fulvescens* var. *elongata*: Glucose is the only sugar fermented. Galactose, sucrose, maltose, mannitol, and α-methyl-D-glucoside are not assimilated. The G+C content of the nuclear DNA of CBS 2594 is 41.8 mol%. Morphologically the varieties are similar. Most strains of the variety *elongata* still sporulate, including the type strain that was isolated in 1913. In contrast, none of the strains of the variety *fulvescens* produced ascospores, which was already noted by Stelling-Dekker (1931).

Origin of the strains belonging to the variety *fulvescens*: CBS 2596, from exudate of an oak, Leningrad, Russia; VKM Y-2618 and Y-2619, from the All-Union Institute of Agricultural Microbiology, Russia, source unknown.

Type strain: CBS 2596, received by CBS from Nadson and Konokotina in 1913.

Origin of strains belonging to the variety *elongata*: From exudates of the following trees in Japan: buckeye (*Aesculus* sp.) (1), alder (*Alnus* sp.) (3), birch (*Betula* sp.) (18), hornbeam (*Carpinus* sp.) (2), dogwood (*Cornus* sp.) (1), *Eurya* sp. (1), beech (*Fagus* sp.) (1), *Prunus* sp. (5), bamboo (*Sinoarundinaria* sp.) (1) (Phaff et al. 1972); from birch exudate, Russia (1); a strain received by CBS in 1927 from Nadson labeled *N. fulvescens* (source unknown) (1); a strain labeled *Nadsonia richteri* (CBS 2593) isolated from hornbeam sap (*Carpinus betulus*) in Czechoslovakia.

Type strain: CBS 2594, isolated by Konokotina in 1913 from birch exudate in the Smolensk area of Russia.

Comments: The two varieties of *N. fulvescens* were originally described as separate species based on a number of differences in physiological properties, some of which were found to be unstable. Golubev et al. (1989) reduced *N. fulvescens* and *N. elongata* to varietal status on the basis of relatively high DNA complementarity (61–71% relative binding). We agree with their interpretation of DNA relatedness values and accept their varietal status as recommended by Golubev et al. (1989).

Comments on the genus

Members of the genus *Nadsonia* were originally isolated from tree exudates in Russia in the early part of the 20th century (Stelling-Dekker 1931). Subsequent ecological studies of yeast habitats revealed that *Nadsonia* species, then known, were found only in tree exudates collected in eastern Europe, Japan, and in some locations of Southeastern USA (Phaff and Starmer 1987). Other areas surveyed extensively for yeast florae from tree exudates and soils indicated that *Nadsonia* species were lacking. Golubev's (1973) discovery of a fundamentally new species of *Nadsonia* in soil from East Falkland Island in the Atlantic Ocean and another isolate of the same species from soil in the Carpathian mountains of eastern Europe (Golubev et al. 1987) revealed an additional soil habitat for members of this genus. Because of the low maximum temperature for growth (22–27°C) of members of *Nadsonia* (Golubev et al. 1989) it is possible that the presence of *Nadsonia* species may have been overlooked in other ecological surveys. The characteristic brown pigment in heavily sporulating cultures of *Nadsonia* has been identified as a melanin compound located in the outer layer of the ascospore wall (Semenova and Golubev 1988). The life cycle of *N. commutata* has been described in detail by Semenova and Golubev (1986).

Note added in proof

Analysis of 5′ end large subunit rDNA nucleotide sequences has demonstrated that *Saccharomycodes sinensis* and *Nadsonia fulvescens* var. *elongata* are conspecific, and that *Schizoblastosporion starkeyi-henricii* is an anamorphic member of the *Nadsonia* clade (C.P. Kurtzman and C.J. Robnett, manuscript in preparation).

41. *Pachysolen* Boidin & Adzet

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal to ellipsoidal and may have one or two small apiculi. Pseudohyphae may be poorly developed or absent. True hyphae are not formed.

Asci are quite unusual and their presence allows immediate recognition of this genus. Generally, a tube grows from a vegetative cell and the end of the tube forms an ascus usually containing four ascospores. The walls of the cell and tube become quite thickened and refractile except for the end bearing the ascospores. The ascospores are hemispheroidal with a narrow ledge at the base.

Glucose is fermented. Nitrate is assimilated. Coenzyme Q-8 is present. Diazonium blue B reaction is negative.

Type species

Pachysolen tannophilus Boidin & Adzet

Species accepted

1. *Pachysolen tannophilus* Boidin & Adzet (1957)

Systematic discussion of the species

41.1. *Pachysolen tannophilus* Boidin & Adzet (1957)

Synonyms:
Hansenula tannophilus (Boidin & Adzet) Campbell (1973)
Pachysolen pelliculatus Boidin & Adzet (1957)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.5–5.0)×(2.0–7.0) µm, and usually have one or two buds. Growth is mucoid to butyrous and tannish-white.

Growth on the surface of assimilation media: Pellicles are formed by some strains.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass exhibits simple undifferentiated pseudohyphae as well as occasional highly branched strands. True hyphae are not formed. Aerobic growth is tannish-white, glistening, mucoid, low convex with margin entire and with a faint smell of esters.

Formation of ascospores: The ascus forms when a vegetative cell produces a stout tube, generally at one end. Depending upon the culture medium, and perhaps other factors, tubes may be quite short or up to 60 µm in length and are straight or curved. The tip of the tube enlarges to form the ascus, and consequently, the tube may be regarded as an ascophore. Asci contain up to four hemispheroidal ascospores that have a narrow ledge at the base. The ascus wall deliquesces, releasing the spores. Once this happens, it is seen that the ascus formed within what appears to be a V-shaped notch at the end of the ascophore. Ascophore walls appear greatly thickened and become quite refractile. This characteristic of highly refractile ascophores bearing terminal asci makes *Pachysolen* readily identifiable under the light microscope even at moderate magnifications (Fig. 127). Asci may be conjugated or unconjugated. The type strain NRRL Y-2460 forms more unconjugated asci than NRRL Y-2461, Y-2462, and Y-2463. Single-spore isolates from NRRL Y-2460 gave sporogenous colonies, therefore, the species appears homothallic (Wickerham 1970b).

Ascospores were observed on YM-, Gorodkowa-, and malt extract agar, after 1 week at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	s	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 8 (Yamada et al. 1973a).

Mol% G+C: 32.6, CBS 4044 and CBS 4045 (BD: C.P. Kurtzman, unpublished); 43.0, CBS 4044 (T_m: Nakase and Komagata 1968a)

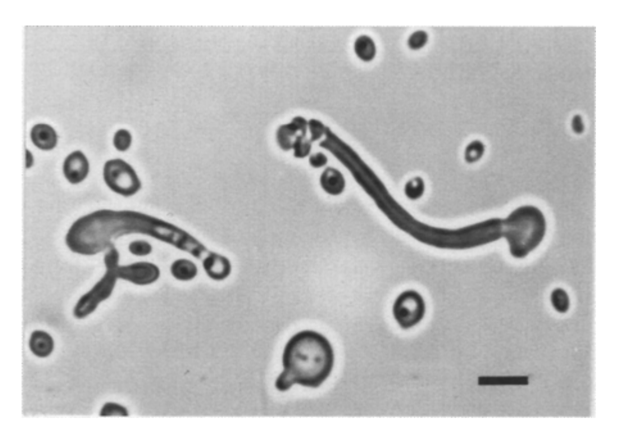


Fig. 127. *P. tannophilus*, CBS 4044. Thick-walled, refractile ascophores bearing terminal asci, after 1 week on YM agar at 25°C. Bar = 5 µm.

Origin of the strains studied: NRRL Y-2460 (IRIC 145, CBS 4044, extract of chestnut (*Castanea vesca*), Ludwigshafen, Germany; NRRL Y-2461 (IRIC 146), NRRL Y-2462 (IRIC 152), NRRL Y-6704 (IRIC 164, CBS 4045), the type strain of *P. penicillatus*, from extracts of acacia (*Acacia mollissima*), Ludwigshafen, Germany; NRRL Y-2463 (IRIC 153), shoe leather, Strasbourg, France.

Type strain: CBS 4044 (NRRL Y-2460), designated by Boidin and Adzet (1957) as the type strain.

Comments on the genus

Pachysolen is a rare and unusual yeast. The only reported isolations are those of Boidin and Adzet (1957), who obtained four strains from wood extracts used in tanning liquors and a fifth strain from shoe leather. Asci of *P. tannophilus* are borne on the tips of refractile tubes which seem to serve as ascophores.

P. tannophilus was originally separated from *P. pelliculatus* because the latter species formed a mat growth on solid media, produced a thin, dry pellicle, had greater development of pseudohyphae, and gave a weaker fermentation of glucose. These differences seem insufficient to differentiate the two species and *P. pelliculatus* is regarded to be a synonym. Wickerham (1970b) showed that a mat form could be selected from *P. tannophilus* by allowing an amoeba to feed on the cultures. The amoeba preferentially ingested mucoid colonies and allowed proliferation of the mat form.

In recent years, *P. tannophilus* has received considerable attention because of its ability to ferment D-xylose, the major pentose of hemicellulosic plant residues (Schneider et al. 1981, Slininger et al. 1982, Kurtzman et al. 1982). Until this discovery, yeasts were not known to produce ethanol from pentoses. Soon after the preceding work, *Pichia stipitis* (Toivola et al. 1984) and *Candida shehatae* (du Preez and van der Walt 1983) were also demonstrated to ferment D-xylose, thus providing three species capable of converting pentoses from biomass to fuel alcohol. Slininger et al. (1987) reviewed the biochemistry and genetics of D-xylose fermentation by *P. tannophilus*. Kurtzman (1990b) estimated phylogenetic relatedness among the three species from extent of rRNA divergence and showed *Pichia stipitis* and *Candida shehatae* to be separate but closely related species whereas *P. tannophilus* was much more distantly related.

42. *Pichia* E.C. Hansen emend. Kurtzman

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Some species may also form arthroconidia. Cells are spheroidal, ellipsoidal, or elongate and occasionally may be tapered, but they are not ogival in shape. Pseudohyphae and true hyphae may be produced by some species.

Asci produce one to four (infrequently more) ascospores that may be hat-shaped (galeate), hemispheroidal, or spheroidal with a ledge. Generally asci are deliquescent, but occasionally they are persistent. Asci are unconjugated, or if conjugated, they may show conjugation between bud and parent or between independent cells. Hyphal or pseudohyphal cells may serve as asci, but they do not become swollen or spindle-like. Asci are not borne on ascophores. Species are homothallic or heterothallic.

Sugars may be fermented. Nitrate may be assimilated. Diazonium blue B reaction is negative.

Type species

Pichia membranifaciens E.C. Hansen

Species accepted

1. *Pichia acaciae* van der Walt (1966)
2. *Pichia alni* (Phaff, M.W. Miller & Miranda) Kurtzman (1984)
3. *Pichia americana* (Wickerham) Kurtzman (1984)
4. *Pichia amethionina* Starmer, Phaff, Miranda & M.W. Miller (1978)
   a. *Pichia amethionina* Starmer, Phaff, Miranda & M.W. Miller var. *amethionina* (1978)
   b. *Pichia amethionina* var. *pachycereana* Starmer, Phaff, Miranda & M.W. Miller (1978)
5. *Pichia amylophila* Kurtzman, Smiley, Johnson, Wickerham & Fuson (1980)
6. *Pichia angophorae* M.W. Miller & Barker (1968)
7. *Pichia angusta* (Teunisson, Hall & Wickerham) Kurtzman (1984)
8. *Pichia anomala* (E.C. Hansen) Kurtzman (1984)
9. *Pichia antillensis* Starmer, Phaff, Tredick, Miranda & Aberdeen (1984)
10. *Pichia barkeri* Phaff, Starmer, Tredick-Kline & Aberdeen (1987)
11. *Pichia besseyi* Kurtzman & Wickerham (1972)
12. *Pichia bimundalis* (Wickerham & Santa María) Kurtzman (1984)
13. *Pichia bispora* (Wickerham) Kurtzman (1984)
14. *Pichia bovis* van Uden & do Carmo-Sousa (1957)
15. *Pichia burtonii* Boidin, Pignal, Lehodey, Vey & Abadie (1964)
16. *Pichia cactophila* Starmer, Phaff, Miranda & M.W. Miller (1978)
17. *Pichia canadensis* (Wickerham) Kurtzman (1984)
18. *Pichia capsulata* (Wickerham) Kurtzman (1984)
19. *Pichia caribaea* Phaff, Starmer, Lachance, Aberdeen & Tredick-Kline (1992)
20. *Pichia castillae* Santa María & García Aser (1970)
21. *Pichia chambardii* (C. Ramírez & Boidin) Phaff (1956)
22. *Pichia ciferrii* (Lodder) Kurtzman (1984)
23. *Pichia delftensis* Beech (1965)
24. *Pichia deserticola* Phaff, Starmer, Tredick & Miranda (1985)
25. *Pichia dryadoides* (D.B. Scott & van der Walt) Kurtzman (1984)
26. *Pichia euphorbiae* van der Walt & Opperman (1983)
27. *Pichia euphorbiiphila* (van der Walt) Kurtzman (1984)
28. *Pichia fabianii* (Wickerham) Kurtzman (1984)
29. *Pichia farinosa* (Lindner) E.C. Hansen (1904)
30. *Pichia fermentans* Lodder (1932)
31. *Pichia finlandica* Kurtzman (1984)
32. *Pichia fluxuum* (Phaff & Knapp) Kreger-van Rij (1964)
33. *Pichia galeiformis* Endo & S. Goto (1987)
34. *Pichia glucozyma* (Wickerham) Kurtzman (1984)

35. *Pichia guilliermondii* Wickerham (1966)
36. *Pichia hampshirensis* Kurtzman (1987)
37. *Pichia haplophila* Shifrine & Phaff (1956)
38. *Pichia heedii* Phaff, Starmer, Miranda & M.W. Miller (1978)
39. *Pichia heimii* Pignal (1970)
40. *Pichia henricii* (Wickerham) Kurtzman (1984)
41. *Pichia holstii* (Wickerham) Kurtzman (1984)
42. *Pichia inositovora* Golubev & Blagodatskaya (1981)
43. *Pichia jadinii* (A. & R. Sartory, Weill & Meyer) Kurtzman (1984)
44. *Pichia japonica* Kurtzman (1987)
45. *Pichia kluyveri* Bedford ex Kudryavtsev (1960)
    a. *Pichia kluyveri* Bedford ex Kudryavtsev var. *kluyveri* (1987)
    b. *Pichia kluyveri* var. *cephalocereana* Phaff, Starmer & Tredick-Kline (1987)
    c. *Pichia kluyveri* var. *eremophila* Phaff, Starmer & Tredick-Kline (1987)
46. *Pichia kodamae* van der Walt & Yarrow (1982)
47. *Pichia lynferdii* (van der Walt & E. Johannsen) Kurtzman (1984)
48. *Pichia media* Boidin, Pignal, Lehodey, Vey & Abadie (1964)
49. *Pichia membranifaciens* (E.C. Hansen) E.C. Hansen (1904)
50. *Pichia methanolica* Makiguchi (1974)
51. *Pichia methylivora* Kumamoto & Seriu (1986)
52. *Pichia mexicana* Miranda, Holzschu, Phaff & Starmer (1982)
53. *Pichia meyerae* van der Walt (1982)
54. *Pichia minuta* (Wickerham) Kurtzman (1984)
    a. *Pichia minuta* (Wickerham) Kurtzman var. *minuta* (1984)
    b. *Pichia minuta* var. *nonfermentans* (Wickerham) Kurtzman (1984)
55. *Pichia mississippiensis* Kurtzman, Smiley, Johnson, Wickerham & Fuson (1980)
56. *Pichia naganishii* K. Kodama (1974)
57. *Pichia nakasei* J.A. Barnett, Payne & Yarrow (1983)
58. *Pichia nakazawae* K. Kodama (1975)
    a. *Pichia nakazawae* K. Kodama var. *nakazawae* (1975)
    b. *Pichia nakazawae* var. *akitaensis* K. Kodama (1975)
59. *Pichia norvegensis* Leask & Yarrow (1976)
60. *Pichia ofunaensis* (Makiguchi & Y. Asai) Kurtzman (1996)
61. *Pichia ohmeri* (Etchells & T.A. Bell) Kreger-van Rij (1964)
62. *Pichia onychis* Yarrow (1965)
63. *Pichia opuntiae* Starmer, Phaff, Miranda, M.W. Miller & Barker (1979)
64. *Pichia pastoris* (Guilliermond) Phaff (1956)
65. *Pichia petersonii* (Wickerham) Kurtzman (1984)
66. *Pichia philodendri* (van der Walt & D.B. Scott) Kurtzman (1984)
67. *Pichia philogaea* van der Walt & E. Johannsen (1975)
68. *Pichia pijperi* van der Walt & Tscheuschner (1957)
69. *Pichia pini* (Holst) Phaff (1956)
70. *Pichia populi* (Phaff, Y. Yamada, Tredick & Miranda) Kurtzman (1984)
71. *Pichia pseudocactophila* Holzschu, Phaff, Tredick & Hedgecock (1983)
72. *Pichia quercuum* Phaff & Knapp (1956)
73. *Pichia rabaulensis* Soneda & Uchida (1971)
74. *Pichia rhodanensis* (C. Ramírez & Boidin) Phaff (1956)
75. *Pichia salicaria* Phaff, M.W. Miller & Spencer (1964)
76. *Pichia scolyti* (Phaff & Yoneyama) Kreger-van Rij (1964)
77. *Pichia segobiensis* Santa María & García Aser (1977)
78. *Pichia silvicola* (Wickerham) Kurtzman (1984)
79. *Pichia spartinae* Ahearn, Yarrow & Meyers (1970)
80. *Pichia stipitis* Pignal (1967)
81. *Pichia strasburgensis* (C. Ramírez & Boidin) Phaff (1956)
82. *Pichia subpelliculosa* Kurtzman (1984)
83. *Pichia sydowiorum* (D.B. Scott & van der Walt) Kurtzman (1984)

84. *Pichia tannicola* F.H. Jacob (1969)
85. *Pichia thermotolerans* (Starmer, Phaff, Miranda, M.W. Miller & Barker) Holzschu, Phaff, Tredick & Hedgecock (1985)
86. *Pichia toletana* (Socias, C. Ramírez & Peláez) Kreger-van Rij (1964)
87. *Pichia trehalophila* Phaff, M.W. Miller & Spencer (1964)
88. *Pichia triangularis* M.Th. Smith & Batenburg-van der Vegte (1986)
89. *Pichia veronae* K. Kodama (1974)
90. *Pichia wickerhamii* (van der Walt) Kreger-van Rij (1964)
91. *Pichia xylosa* Phaff, M.W. Miller & Shifrine (1956)

Key to species

See Tables 31–35.

1. a Hexadecane is assimilated → 2
   b Hexadecane is not assimilated → 18

2(1). a Sucrose is assimilated → 3
   b Sucrose is not assimilated → 14

3(2). a Galactose is assimilated → 4
   b Galactose is not assimilated .... *P. spartinae*: p. 341

4(3). a Raffinose is assimilated → 5
   b Raffinose is not assimilated → 10

5(4). a Soluble starch is assimilated .... *P. burtonii*: p. 293
   b Soluble starch is not assimilated → 6

6(5). a L-Arabinose is assimilated → 7
   b L-Arabinose is not assimilated .... *P. ohmeri*: p. 329

7(6). a Erythritol is assimilated → 8
   b Erythritol is not assimilated .... *P. guilliermondii*: p. 308

8(7). a Growth in vitamin-free medium .... *P. heimii*: p. 311
   b Absence of growth in vitamin-free medium → 9

9(7). a Trehalose fermentation is strong .... *P. mexicana*: p. 322
   b Trehalose fermentation is weak .... *P. scolyti*: p. 339

Table 31
Key characters of *Pichia* species that assimilate hexadecane

Species	Fermentation[a,b]				Assimilation[a,b]										Vfree[a,b]
	G	Ga	Ma	Tr	Ga	Su	Ma	Mel	Raf	Mz	St	L-Ar	Rh	Er	
Pichia acaciae	+	v	+	+	+	–	+	–	–	–	–	+	–	+	–
P. burtonii	+	v	×	+	+	+	+	–	+	v	+	v	–	+	+
P. castillae	–	–	–	–	+	–	+	+	+	–	–	+	–	+	–
P. farinosa	+	v	–	v	+	–	v	–	–	–	v	v	–	+	v
P. guilliermondii	+	v	–	+	+	+	+	+	+	+	–	+	v	–	–
P. haplophila	–	–	–	–	+	–	–	–	–	–	–	+	–	+	–
P. heimii	+	+	v	+	+	+	+	–	+	+	–	+	+	+	+
P. media	–	–	–	–	+	–	+	–	–	–	v	+	–	+	–
P. mexicana	+	×	–	+	+	+	+	v	+	+	–	+	+	+	–
P. nakazawae var. *nakazawae*	+	+	w	+	+	+	+	–	–	+	+	+	+	–	–
P. nakazawae var. *akitaensis*	+	–	w	w	+	+	+	–	–	+	+	+	–	+	–
P. ohmeri	+	×	v	v	+	+	+	–	+	–	–	–	–	–	–
P. philogaea	+	w	v	+	+	+	+	–	–	+	–	+	–	+	–
P. scolyti	w	w	v	w	+	+	+	+	+	+	–	+	+	+	–
P. segobiensis	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–
P. spartinae	+	–	v	–	–	+	+	–	–	+	–	–	–	–	–
P. stipitis	+	w	+	+	+	+	+	–	–	+	+	v	+	+	–

[a] Abbreviations: G, glucose; Ga, galactose; Su, sucrose; Ma, maltose; Tr, trehalose; Mel, melibiose; Raf, raffinose; Mz, melezitose; St, soluble starch; L-Ar, L-arabinose; Rh, L-rhamnose; Er, erythritol; Vfree, vitamin-free medium.
[b] Symbols: +, strong positive; ×, weak or strong; w, weak; v, variable; –, negative.

Table 32
Key characters of *Pichia* species that do not assimilate hexadecane but do assimilate *N*-acetyl-D-glucosamine

Species	Glucose fermentation	Assimilation[a,b]										
		Ga	L-Sor	Su	Ma	Mel	Raf	D-Xy	L-Ar	D-Gm	Rbl	Cit
Pichia angophorae	+	–	–	+	+	–	–	+	+	+	+	×
P. barkeri	+	–	–	–	–	–	–	v	–	+	–	–
P. capsulata	+	–	–	–	+	–	–	+	v	+	+	–
P. delftensis	w/–	–	–	–	–	–	–	–	–	–	+	–
P. fermentans	+	–	–	–	–	–	–	+	–	+	–	+
P. galeiformis	–	–	–	–	–	–	–	–	–	–	–	–
P. holstii	+	+	+	+	+	–	–	+	+	+	+	+
P. inositovora	–	+	+	+	+	–	–	+	–	–	+	+
P. kluyveri var. *kluyveri*	+	–	–	–	–	–	–	v	–	+	–	v
P. kluyveri var. *cephalocereana*	s	–	–	–	–	–	–	–	–	+	–	w
P. kluyveri var. *eremophila*	w/–	–	–	–	–	–	–	–	–	+	–	×
P. membranifaciens[c]	w/–	–	v	–	–	–	–	v	–	v	–	v
P. methylivora	–	–	+	+	–	–	+	+	+	+	+	+
P. naganishii	+	+	–	+	+	–	–	+	+	+	+	–
P. ofunaensis	w	+	+	v	v	+	v	+	+	+	+	+
P. tannicola	×	+	–	–	–	+	–	+	+	+	+	×
P. toletana	w	–	–	+	+	–	–	+	–	–	–	w
P. triangularis	w/–	+	–	+	+	–	–	+	+	–	+	v
P. xylosa	w/–	–	–	+	+	–	–	+	–	v	–	v

[a] Abbreviations: Ga, galactose; L-Sor, L-sorbose; Su, sucrose; Ma, maltose; Mel, melibiose; Raf, raffinose; D-Xy, D-xylose; L-Ar, L-arabinose; D-Gm, D-glucosamine; Rbl, ribitol; Cit, citrate.
[b] Symbols: +, strong positive; s, slow, positive; l, latent, positive; w, weak; w/–, weak or negative; ×, weak or strong; v, variable; –, negative.
[c] See Comments (p. 320) for further discussion on separation of *P. membranifaciens* from phenotypically similar taxa.

10(4). a Melezitose is assimilated → 11
b Melezitose is not assimilated .... *P. segobiensis*: p. 340
11(10). a L-Rhamnose is assimilated → 12
b L-Rhamnose is not assimilated → 13
12(11). a Galactose fermentation is strong; maltose fermentation is weak .... *P. nakazawae* var. *nakazawae*: p. 327
b Galactose fermentation is weak; maltose fermentation is strong .... *P. stipitis*: p. 342
13(11). a Soluble starch is assimilated .... *P. nakazawae* var. *akitaensis*: p. 327
b Soluble starch is not assimilated .... *P. philogaea*: p. 333
14(2). a Glucose is fermented → 15
b Glucose is not fermented → 16
15(14). a Maltose is fermented .... *P. acaciae*: p. 282
b Maltose is not fermented .... *P. farinosa*: p. 304
16(14). a Melibiose is assimilated .... *P. castillae*: p. 298
b Melibiose is not assimilated → 17
17(16). a Maltose is assimilated .... *P. media*: p. 318
b Maltose is not assimilated .... *P. haplophila*: p. 310
18(1). a *N*-Acetyl-D-glucosamine is assimilated → 19
b *N*-Acetyl-D-glucosamine is not assimilated → 33
19(18). a Galactose is assimilated → 20
b Galactose is not assimilated → 25
20(19). a L-Sorbose is assimilated → 21
b L-Sorbose is not assimilated → 23
21(20). a Melibiose is assimilated .... *P. ofunaensis*: p. 328
b Melibiose is not assimilated → 22
22(21). a L-Arabinose is assimilated .... *P. holstii*: p. 312
b L-Arabinose is not assimilated .... *P. inositovora*: p. 313

Table 33
Key characters of *Pichia* species that assimilate methanol but do not assimilate *N*-acetyl-D-glucosamine

Species	Glucose fermentation	Assimilation[a]							
		L-Sorbose	Maltose	Cellobiose	Trehalose	D-Arabinose	Erythritol	Galactitol	Nitrate
Pichia angusta	+	v	+	v	+	v	+	v	+
P. finlandica	–	+	–	–	+	+	+	–	+
P. glucozyma	+	–	–	+	+	–	+	–	+
P. henricii	–	–	–	+	v	–	+	–	+
P. kodamae	+	v	–	+	–	–	+	–	–
P. methanolica	+	v	–	+	+	×	+	+	–
P. minuta var. *minuta*	×	–	–	+	+	v	–	–	v
P. minuta var. *nonfermentans*	–	–	–	+	+	–	–	–	+
P. pastoris	+	–	–	–	+	–	–	–	–
P. philodendri	–	+	–	–	+	–	+	–	+
P. pini	v	v	–	+	+	v	+	–	–
P. trehalophila	+	+	–	–	+	v	+	–	–

[a] Symbols: +, strong positive; ×, weak or strong; v, variable; –, negative.

23(20). a Sucrose is assimilated → 24
b Sucrose is not assimilated ... *P. tannicola*: p. 345
24(23). a D-Glucosamine is assimilated ... *P. naganishii*: p. 325
b D-Glucosamine is not assimilated ... *P. triangularis*: p. 348
25(19). a Sucrose is assimilated → 26
b Sucrose is not assimilated → 28
26(25). a Raffinose is assimilated ... *P. methylivora*: p. 321
b Raffinose is not assimilated → 27
27(26). a L-Arabinose is assimilated ... *P. angophorae*: p. 286
b L-Arabinose is not assimilated ... *P. toletana*: p. 346
P. xylosa: p. 350
28(25). a Maltose is assimilated ... *P. capsulata*: p. 296
b Maltose is not assimilated → 29
29(28). a Ribitol is assimilated ... *P. delftensis*: p. 299
b Ribitol is not assimilated → 30
30(29). a Glucose is strongly fermented → 31
b Glucose fermentation is slow, weak or absent → 32
31(30). a Citrate and D-xylose are strongly assimilated ... *P. fermentans*: p. 305
b Citrate is not assimilated; D-xylose assimilation is weak or absent; no killer activity against *Candida glabrata* .. *P. barkeri*: p. 289
c Citrate and D-xylose assimilation are weak or absent; killer activity against *Candida glabrata* .... *P. kluyveri* var. *kluyveri*: p. 315
32(30). a Glucose is not fermented; D-glucosamine and citrate are not assimilated ... *P. galeiformis*: p. 307
P. membranifaciens: p. 319
b Glucose fermentation is slow and usually late; D-glucosamine and citrate are assimilated .. *P. kluyveri* var. *cephalocereana*: p. 316
c Glucose fermentation is weak or absent; D-glucosamine and citrate are assimilated ... *P. kluyveri* var. *eremophila*: p. 316
P. membranifaciens: p. 319
d Glucose fermentation is weak or absent; L-sorbose is assimilated ... *P. membranifaciens*: p. 319
33(18). a Methanol assimilation is weak or strong → 34
b Methanol is not assimilated → 45
34(18). a Maltose is assimilated ... *P. angusta*: p. 286
b Maltose is not assimilated → 35
35(34). a Cellobiose is assimilated → 36
b Cellobiose is not assimilated → 42
36(35). a Erythritol is assimilated → 37
b Erythritol is not assimilated → 41
37(36). a Galactitol is assimilated ... *P. methanolica*: p. 321
b Galactitol is not assimilated → 38
38(37). a Nitrate is assimilated → 39
b Nitrate is not assimilated → 40
39(38). a Glucose is fermented ... *P. glucozyma*: p. 307
b Glucose is not fermented ... *P. henricii*: p. 311

Table 34
Key characters of *Pichia* species that assimilate raffinose but do not assimilate *N*-acetyl-D-glucosamine

Species	Assimilation[a]							Growth in vitamin-free medium[a]
	Galactose	Melibiose	Soluble starch	L-Rhamnose	Erythritol	Ribitol	Nitrate	
Pichia anomala	v	–	+	–	+	v	+	+
P. ciferrii	+	–	+	×	+	+	+	+
P. fabianii	–	–	+	–	–	–	+	–
P. jadinii	–	–	–	–	–	–	+	+
P. lynferdii	+	–	–	–	+	+	+	+
P. onychis	–	–	–	–	–	–	–	–
P. petersonii	–	–	–	+	–	–	+	–
P. rabaulensis	–	–	–	v	–	+	–	–
P. strasburgensis	+	–	–	+	–	+	–	–
P. subpelliculosa	v	–	v	–	+	v	+	–
P. sydowiorum	+	+	v	+	+	+	+	+
P. veronae	–	–	–	+	–	–	–	–

[a] Symbols: +, strong positive; ×, weak or strong; v, variable; –, negative.

40(38). a Trehalose is assimilated .... *P. pini*: p. 334
b Trehalose is not assimilated .... *P. kodamae*: p. 317
41(36). a Glucose is fermented .... *P. minuta* var. *minuta*: p. 324
b Glucose is not fermented .... *P. minuta* var. *nonfermentans*: p. 324
42(35). a Glucose is fermented → 43
b Glucose is not fermented → 44
43(42). a L-Sorbose is assimilated .... *P. trehalophila*: p. 347
b L-Sorbose is not assimilated .... *P. pastoris*: p. 331
44(42). a D-Arabinose is assimilated .... *P. finlandica*: p. 305
b D-Arabinose is not assimilated .... *P. philodendri*: p. 333
45(33). a Raffinose is assimilated → 46
b Raffinose is not assimilated → 57
46(45). a Melibiose is assimilated .... *P. sydowiorum*: p. 344
b Melibiose is not assimilated → 47
47(46). a Erythritol is assimilated → 48
b Erythritol is not assimilated → 51
48(47). a Growth in vitamin-free medium → 49
b Absence of growth in vitamin-free medium .... *P. subpelliculosa*: p. 344
49(48). a Soluble starch is assimilated → 50
b Soluble starch is not assimilated .... *P. lynferdii*: p. 317
50(49). a L-Rhamnose is assimilated .... *P. ciferrii*: p. 299
b L-Rhamnose is not assimilated .... *P. anomala*: p. 287
51(47). a Galactose is assimilated .... *P. strasburgensis*: p. 343
b Galactose is not assimilated → 52
52(51). a Soluble starch is assimilated .... *P. fabianii*: p. 303
b Soluble starch is not assimilated → 53
53(52). a Growth in vitamin-free medium .... *P. jadinii*: p. 314
b Absence of growth in vitamin-free medium → 54
54(53). a Ribitol is assimilated .... *P. rabaulensis*: p. 337
b Ribitol is not assimilated → 55
55(54). a Nitrate is assimilated .... *P. petersonii*: p. 332
b Nitrate is not assimilated → 56
56(55). a L-Rhamnose is assimilated .... *P. veronae*: p. 348
b L-Rhamnose is not assimilated .... *P. onychis*: p. 330
57(45). a Growth in vitamin-free medium .... *P. dryadoides*: p. 301
b Absence of growth in vitamin-free medium → 58
58(57). a Galactose is assimilated → 59
b Galactose is not assimilated → 60
59(58). a Glucose is fermented .... *P. silvicola*: p. 340
b Glucose is not fermented .... *P. chambardii*: p. 298

60(58). a Sucrose is assimilated → 61
b Sucrose is not assimilated → 74
61(60). a Melezitose is assimilated → 62
b Melezitose is not assimilated .... *P. meyerae*: p. 323
62(61). a Soluble starch is assimilated → 63
b Soluble starch is not assimilated → 64
63(62). a Salicin is assimilated .... *P. bovis*: p. 292
b Salicin is not assimilated .... *P. amylophila*: p. 285
64(62). a Nitrate is assimilated → 65
b Nitrate is not assimilated → 69
65(64). a D-Arabinose is assimilated .... *P. bimundalis*: p. 291
b D-Arabinose is not assimilated → 66
66(65). a L-Rhamnose is assimilated → 67
b L-Rhamnose is not assimilated .... *P. euphorbiiphila*: p. 302
67(66). a Growth at 37°C .... *P. canadensis*: p. 295
b Absence of growth at 37°C → 68
68(67). a True hyphae are formed; early (1–10 days) weak or strong fermentation of glucose .... *P. americana*: p. 283
b True hyphae are formed; glucose fermentation is absent or weak and late (more than 10 days) .... *P. bispora*: p. 291
c True hyphae are not formed; glucose fermentation is absent .... *P. alni*: p. 282
69(64). a Glucose is fermented → 70
b Glucose is not fermented .... *P. canadensis*: p. 295
70(69). a 2-Keto-D-gluconate is assimilated .... *P. wickerhamii*: p. 349
b 2-Keto-D-gluconate is not assimilated → 71
71(70). a L-Arabinose is assimilated → 72
b L-Arabinose is not assimilated → 73
72(71). a True hyphae are formed; sucrose is weakly fermented .... *P. euphorbiae*: p. 301
b True hyphae are formed; sucrose is not fermented .... *P. mississippiensis*: p. 325
c True hyphae are not formed .... *P. bovis*: p. 292
73(71). a Growth at 37°C; true hyphae are often formed; gelatin is not liquified .... *P. rhodanensis*: p. 338
b Growth at 37°C; true hyphae are not formed; gelatin is weakly liquified .... *P. japonica*: p. 315
c Absence of growth at 37°C .... *P. hampshirensis*: p. 309
74(60). a Mannitol is assimilated → 75
b Mannitol is not assimilated → 85
75(74). a Glucose is fermented → 76
b Glucose is not fermented → 79
76(75). a L-Sorbose is assimilated .... *P. pijperi*: p. 334
b L-Sorbose is not assimilated → 77
77(76). a Cellobiose is assimilated .... *P. quercuum*: p. 337
b Cellobiose is not assimilated → 78
78(77). a D-Glucitol is assimilated .... *P. besseyi*: p. 290
b D-Glucitol is not assimilated .... *P. caribaea*: p. 297
79(75). a D-Xylose is assimilated → 80
b D-Xylose is not assimilated → 81
80(79). a Nitrate is assimilated .... *P. populi*: p. 335
b Nitrate is not assimilated .... *P. salicaria*: p. 338
81(79). a Citrate is assimilated → 82
b Citrate is not assimilated → 83
82(81). a Cellobiose is not assimilated; salicin assimilation is variable; isolated from columnar cacti, West Indies .... *P. antillensis*: p. 288
b Cellobiose assimilation is variable; salicin is assimilated; isolated from *Opuntia* cacti, Australia .... *P. opuntiae*: p. 330
83(81). a Cellobiose is assimilated .... *P. thermotolerans*: p. 346
b Cellobiose is not assimilated → 84
84(83). a D-Glucitol is assimilated .... *P. fluxuum*: p. 306
b D-Glucitol is not assimilated .... *P. amethionina* var. *pachycereana*: p. 284
85(74). a D-Glucosamine is assimilated → 86
b D-Glucosamine is not assimilated → 88
86(85). a Cellobiose is assimilated .... *P. norvegensis*: p. 328
b Cellobiose is not assimilated → 87
87(86). a D-Xylose is assimilated weakly or latently; asci form two hat-shaped ascospores .... *P. cactophila*: p. 294
b D-Xylose is not assimilated; asci form four hat-shaped ascospores .... *P. pseudocactophila*: p. 336
88(85). a Growth in osmotic medium (10% sodium chloride + 5% glucose) .... *P. nakasei*: p. 326
b Absence of growth in osmotic medium → 89
89(88). a D-Gluconate is assimilated .... *P. amethionina* var. *amethionina*: p. 284
b D-Gluconate is not assimilated → 90
90(89). a D-Xylose is assimilated .... *P. heedii*: p. 310
b D-Xylose is not assimilated .... *P. deserticola*: p. 300

Table 35

Key characters of *Pichia* species that do not assimilate hexadecane, methanol, raffinose or *N*-acetyl-D-glucosamine

Species	Ferment.[a,b]		Assimilation[a,b]																	Other				
	G	Su	Ga	L-Sor	Su	Ce	Mz	St	D-Xy	L-Ar	D-Ar	L-Rh	D-Gm	D-Gcl	Sa	D-Glt	Cit	2-Keto	NO_3	Vfr	Osm	37°	Gel	TrH
Pichia alni	−	−	−	−	+	+	+	−	+	−	−	+	−	+	+	+	+	−	+	−	v	−	−	−
P. americana	×	−	−	−	+	+	+	−	+	−	−	+	−	+	+	×	+	v	+	−	−	−	−	−
P. amethionina var. *amethionina*	−	−	−	−	−	−	−	−	v	−	−	−	−	−	−	+	−	v	−	−	−	+	−	−
P. amethionina var. *pachycereana*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−
P. amylophila	+	−	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	−	−	−	+	−	+
P. antillensis	−	−	−	−	−	−	−	−	−	−	−	+	−	−	v	−	+	−	−	−	−	−	−	−
P. besseyi	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−
P. bimundalis	+	−	−	−	+	+	+	−	+	v	+	+	−	+	+	+	+	v	+	−	−	+	−	+
P. bispora	v	−	−	−	+	+	+	−	+	v	−	+	−	×	×	+	+	−	+	−	−	−	−	+
P. bovis	+	v	−	−	+	+	+	v	+	+	−	v	−	+	+	+	+	−	−	−	−	+	−	−
P. cactophila	v	−	−	−	−	−	−	−	×	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−
P. canadensis	−	−	−	−	+	+	+	−	+	−	−	×	−	+	+	+	+	−	v	−	−	+	−	+
P. caribaea	+	−	−	−	−	−	−	−	v	−	−	−	−	−	−	+	−	−	−	−	−	v	−	−
P. chambardii	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	−	w	−	−	−	−	−	−	−
P. deserticola	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
P. dryadoides	−	−	−	−	−	+	−	−	v	−	−	−	−	+	−	+	+	−	+	+	−	v	−	−
P. euphorbiae	+	w	−	−	+	+	+	−	+	+	−	+	−	+	+	+	+	−	−	−	−	v	−	+
P. euphorbiiphila	+	−	−	−	+	+	+	−	+	−	−	−	−	+	+	+	+	−	+	−	v	+	−	−
P. fluxuum	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
P. hampshirensis	+	−	−	−	+	+	+	−	+	−	−	+	−	+	+	v	+	−	−	−	−	−	−	−
P. heedii	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	v	−	−	−	−	+	−	−
P. japonica	×	−	−	−	+	+	+	−	+	−	−	+	−	+	+	×	×	−	−	−	−	+	w	−
P. meyerae	+	−	−	−	+	+	−	−	+	−	−	+	−	v	+	+	+	−	−	−	−	+	−	v
P. mississippiensis	+	−	−	−	+	+	+	−	+	+	v	v	−	+	v	+	v	−	−	−	−	+	−	+
P. nakasei	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	×	−	v	−
P. norvegensis	+	−	−	−	−	+	−	−	−	−	−	−	+	−	+	−	w	−	−	−	+	+	−	−
P. opuntiae	−	−	−	−	−	v	−	−	−	−	−	+	−	−	+	−	×	−	−	−	−	−	−	−
P. pijperi	+	−	−	+	−	+	−	−	+	−	−	−	−	+	+	−	v	−	−	−	−	v	−	−
P. populi	−	−	−	−	−	+	−	−	+	−	−	+	−	+	+	+	−	−	+	−	−	−	−	−

continued on next page

Table 35, *continued*

Species	Ferment.[a,b]		Assimilation[a,b]																	Other				
	G	Su	Ga	L-Sor	Su	Ce	Mz	St	D-Xy	L-Ar	D-Ar	L-Rh	D-Gm	D-Gcl	Sa	D-Glt	Cit	2-Keto	NO_3	Vfr	Osm	37°	Gel	TrH
P. pseudocactophila	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	v	–	–	–	–	+	v	–
P. quercuum	w	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	×	–	–	–	–	×	–	–
P. rhodanensis	+	v	–	–	+	+	+	–	+	–	–	+	–	+	v	+	+	–	–	–	–	+	–	v
P. salicaria	–	–	–	–	–	+	–	–	+	–	–	+	–	+	+	+	+	+	–	–	–	+	–	–
P. silvicola	+	–	+	v	v	+	v	–	+	+	–	+	–	+	+	+	v	–	+	–	–	v	–	v
P. thermotolerans	–	–	–	–	–	+	–	–	–	–	–	v	–	v	+	–	–	–	–	–	–	+	–	–
P. wickerhamii	+	–	–	–	+	+	+	–	+	v	–	+	–	+	+	+	+	×	–	–	×	+	v	v

[a] Abbreviations: Ferment., fermentation; G, glucose; Su, sucrose.
Assimilation: Ga, galactose; L-Sor, L-sorbose; Su, sucrose; Ce, cellobiose; Mz, melezitose; St, soluble starch; D-Xy, D-xylose; L-Ar, L-arabinose; D-Ar, D-arabinose; L-Rh, L-rhamnose; D-Gm, D-glucosamine; D-Gcl, D-glucitol; Sa, salicin; D-Glt, D-gluconate; Cit, citrate; 2-Keto, 2-keto-D-gluconate; NO_3, nitrate.
Other: Vfr, growth in vitamin-free medium; Osm, growth in osmotic medium (10% sodium chloride + 5% glucose); 37°, growth at 37°C; Gel, gelatin liquefaction; TrH, presence of true hyphae.
[b] Symbols: +, strong positive; ×, weak or strong; w, weak; v, variable; l, latent; –, negative.

Systematic discussion of the species

42.1. *Pichia acaciae* van der Walt (1966b)

Synonym:
Yamadazyma acaciae (van der Walt) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to elongate, (1.5–5.5)×(2.5–11) µm, and occur singly, in pairs or in small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows moderately well-developed pseudohyphae but no true hyphae. Aerobic growth is butyrous, dull, grayish-white, and has a somewhat wrinkled surface and a lobed margin.

Formation of ascospores: Asci show conjugation between a cell and its bud, or rarely, conjugation between independent cells. Two to four hat-shaped ascospores are produced in each ascus, and they are released soon after formation (Fig. 128).

Ascospores were observed on YM and 5% malt extract agars after 5–8 days at 15°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	w/–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+/w
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+/w	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 5656 (Billon-Grand 1989).
Mol% G+C: 46.0 (J.B. Fiol, cited by Billon-Grand 1981).

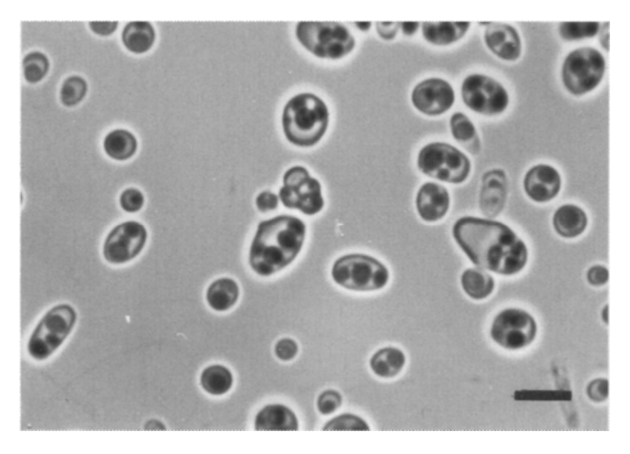


Fig. 128. *P. acaciae*, CBS 5656. Ascosporogenous culture after 2 weeks, 15°C, on 5% malt extract agar. Bar = 5 µm.

Origin of the strains studied: Frass of Buprestidae beetle larvae infesting acacia (*Acacia nilotica* var. *kraussiana*), South Africa (1); tunnels of the beetle *Sinoxylon ruficorne* in *Combretum apiculatum*, South Africa (1).

Type strain: CBS 5656 (NRRL Y-7117), from frass of Buprestidae larvae.

Comments:
Some differences in carbon assimilation for *P. acaciae* were noted among van der Walt's (1966b) original description, Kreger-van Rij's (1970c) study and the present treatment. Consequently, growth on soluble starch and ribitol might be variable rather than positive as given in this study. Additionally, the type strain gave a late, weak reaction on D-xylose rather than a negative reaction as previously reported. *P. acaciae* is phenotypically quite similar to *P. media*, and the two differ only because *P. media* is nonfermentative.

42.2. *Pichia alni* (Phaff, M.W. Miller & Miranda) Kurtzman (1984a)

Synonym:
Hansenula alni Phaff, M.W. Miller & Miranda (1979)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal and measure (2.3–4.8)×(2.9–5.7) µm. They occur singly, in pairs, or in small clusters. Growth is butyrous, glistening, and cream-colored to white.

Growth on the surface of assimilation media: Thin, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass is sparse and pseudohyphae are poorly developed. True hyphae are not produced. Aerobic growth is tannish-white, smooth to faintly striated, glistening and with a slightly depressed center. Colony margins are entire to broadly lobed. A faint acidic odor is present.

Formation of ascospores: This species is heterothallic and has been isolated from nature only in the haploid state. Ascospores develop in diploid cells resulting from

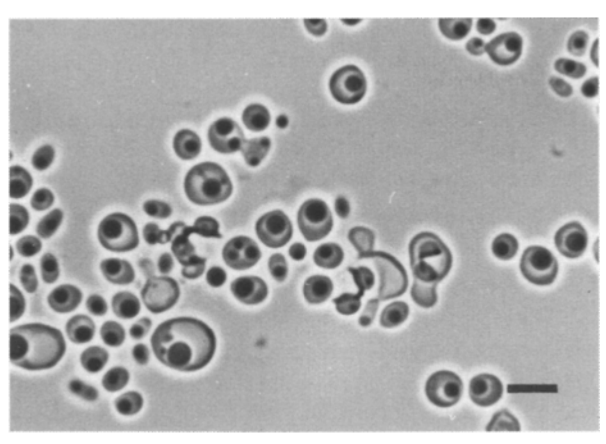


Fig. 129. *P. alni*, CBS 6986×CBS 6987. Ascosporogenous culture after 10 days, 25°C, on 5% malt extract agar. Bar = 5 µm.

conjugation between complementary mating types. Infrequently, the zygote itself will serve as the ascus. There are two to four hat-shaped ascospores per ascus, and asci are deliquescent (Fig. 129).

Ascospores were observed on 5% malt extract agar after 5–7 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	w/–	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	w/–		

Co-Q: 7, CBS 6986 (Y. Yamada and H.J. Phaff, personal communication).

Mol% G + C: 41.8–42.0, CBS 6986 and 2 additional strains (BD: Phaff et al. 1979).

Origin of the strains studied: Flux from alder (*Alnus rubra* Bongard), British Columbia, Canada (2); flux from *A. rubra*, Washington, U.S.A. (1).

Complementary mating types: NRRL Y-11625 (CBS 6986, type strain) and NRRL Y-11626 (CBS 6987).

Type strain: CBS 6986 (UCD-FST 68-928A, NRRL Y-11625), isolated by Phaff et al. (1979) from flux of *A. rubra*, British Columbia, Canada.

Comments: Phaff et al. (1979) reported limited conjugation between the mating types of *P. alni* and *P. canadensis* (*Hansenula wingei*), but ascospores were not formed. In an accompanying study, Fuson et al. (1979) showed the two species to exhibit only 6% nDNA base sequence complementarity, thus confirming them as separate taxa. All isolates of *P. alni* have been from deciduous trees, whereas *P. canadensis* occurs exclusively on coniferous trees. Nonetheless, it appears that the two species have recently evolved from a common ancestor.

Minor differences in carbon assimilation were noted between the original description and the present study. Phaff et al. (1979) reported weak growth on soluble starch and no growth on ribitol. In the present study, none of the strains grew on soluble starch, and one of the strains (NRRL Y-11626) gave a strong but latent reaction on ribitol.

42.3. *Pichia americana* (Wickerham) Kurtzman (1984a)

Synonyms:

Hansenula bimundalis Wickerham & Santa María var. *americana* Wickerham (1965b)

Hansenula americana (Wickerham) Kurtzman (1984b)

Candida bimundalis Wickerham & Santa María var. *americana* Wickerham (1965b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.8–4.0)×(1.9–4.2) µm, and single or in pairs. Growth is butyrous and light cream colored.

Growth on the surface of assimilation media: Only occasional strains form pellicles.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and moderately well differentiated pseudohyphae as well as true hyphae. Aerobic growth is white to tannish-white, butyrous, and glistening or dull. Hyphae or pseudohyphae may be found along the edges of colonies. An ester-like odor is produced.

Formation of ascospores: This species is heterothallic and only haploid strains have been isolated from nature. Following conjugation between complementary mating types, zygotes usually serve as asci, although occasional diploid cells bud from the zygote and become asci. The ascospores are hat-shaped and there are usually four per ascus. Asci are deliquescent.

Ascospores were observed on 5% malt extract agar after 5–20 days at 25°C, following pairing of complementary mating types.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+/w
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+/w
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	w/–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 5644 (Yamada et al. 1973a).

Mol% G+C: 42.9, 43.6, 44.0, CBS 5644 (T_m: Nakase and Komagata 1971b; BD: Fuson et al. 1979; BD: Kurtzman 1984b); 43.2, NRRL YB-2444 (BD: Kurtzman 1984b).

Origin of the strains studied: Frass from a Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), California, U.S.A. (3); frass from a ponderosa pine (*Pinus ponderosa* Laws.), New Mexico, U.S.A. (2).

Complementary mating types: NRRL Y-2156 (CBS 5644) and NRRL Y-2157 (CBS 5645).

Type strain: CBS 5644 (NRRL Y-2156), one of the strains from frass from Jeffrey pine, California, U.S.A.

Comments: In 1965, Wickerham (1965b) described the species *Hansenula bimundalis* and the variety *americana*. Strains of *H. bimundalis* var. *bimundalis* are associated with coniferous trees of Europe and Asia, whereas strains of *H. bimundalis* var. *americana* are found with coniferous trees of southwestern United States. The variety *bimundalis* assimilates D-arabinose and grows at 37°C, but the variety *americana* does not. Wickerham also noted that hyphae of the variety *bimundalis* are about twice as long as hyphae of the variety *americana*. In mating studies, zygotes formed from intervarietal crosses, but ascospores were never produced.

Fuson et al. (1979) examined the nDNA of certain tree-inhabiting species of *Hansenula* (=*Pichia*) and noted that the G+C content of *H. bimundalis* var. *americana* was 2.5% higher than that of *H. bimundalis* var. *bimundalis*. On the basis of previous experience, this was highly suggestive that the varieties represented distinct species. Kurtzman (1984b) demonstrated by nDNA reassociation studies that the two varieties showed only 21% base sequence complementarity. This relatively low nDNA relatedness coupled with the infertility of intervarietal crosses provided evidence for elevation of *P. bimundalis* var. *americana* to species status.

42.4. *Pichia amethionina* Starmer, Phaff, Miranda & M.W. Miller (1978b)

This species has two varieties:

***Pichia amethionina* Starmer, Phaff, Miranda & M.W. Miller var. *amethionina* (1978b)**

***Pichia amethionina var. pachycereana* Starmer, Phaff, Miranda & M.W. Miller (1978b)**

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (1.2–4.0)×(3.0–9.0) µm, and occur singly, in pairs, in short branched chains and in stellate clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows no true hyphae and only a few rudimentary pseudohyphae. Aerobic growth is white in color, smooth, glistening, and butyrous with an irregular and finely serrate margin.

Formation of ascospores: This species is heterothallic and asci are either unconjugated if formed by diploid cells or conjugated when produced by the pairing of complementary mating types. Each ascus contains one to four hat-shaped ascospores which are readily released at maturity.

Ascospores were observed on 5% malt extract agar after 3–4 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 6940 (Billon-Grand 1985).

Mol% G+C: 33.0–33.1, CBS 6940 and 5 additional strains of the

variety *amethionina*; 32.8–33.5, CBS 6943 and 3 additional strains of the variety *pachycereana* (BD: Starmer et al. 1978b).

Supplementary description of *P. amethionina* var. *pachycereana*: The variety *pachycereana* assimilates D-mannitol, but does not assimilate D-xylose, thereby providing a means to separate it from the variety *amethionina*.

Origin of the strains belonging to the variety *amethionina*: From cina cactus (*Rathbunia alamosensis*) (1) and agria cactus (*Macherocereus gummosus*) (2), Mexico.

Complementary mating types: NRRL Y-10979 (CBS 6942) and NRRL Y-10980 (CBS 6941), derived from the type strain by Starmer et al. (1978b).

Type strain: CBS 6940 (NRRL Y-10978).

Origin of the strains belonging to the variety *pachycereana*: From cardon cactus (*Pachycereus pringlei*) (2) and agria cactus (*Macherocereus gummosus*) (1), Mexico.

Complementary mating types: NRRL Y-10982 (CBS 6945) and NRRL Y-10983 (CBS 6944), derived from the type strain by Starmer et al. (1978b).

Type strain: CBS 6943 (NRRL Y-10981).

Comments: Starmer et al. (1978b, 1979) observed no mating between *P. amethionina* and *P. delftensis, P. heedii* or *P. opuntiae* despite their phenotypic similarity, and it appears that these species are biologically distinct. *P. amethionina* and *P. caribaea* seem unique among the yeasts because all strains require an exogenous source of methionine or cysteine (Starmer et al. 1978b, Phaff et al. 1992). Shen and Lachance (1993) have compiled the published data on extent of nDNA complementarity among closely related cactus yeasts.

42.5. *Pichia amylophila* Kurtzman, Smiley, Johnson, Wickerham & Fuson (1980a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are single, or rarely, clustered, and spheroidal, ellipsoidal, or elongate, (2.0–5.5)×(3.0–14.0) μm. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed but rings develop occasionally.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and highly branched pseudohyphae as well as occasional true hyphae. The true hyphae show no evidence of a dolipore septum when viewed under the light microscope. Aerobic growth is cream colored, dull, and moist with a serrate margin and a slightly raised center. A faint, ester-like odor is produced.

Formation of ascospores: Diploid cells convert to asci and usually form two hat-shaped ascospores. Asci are generally free but may form on pseudohyphae where they are sessile or attached by short sterigmata. At maturity, the asci become deliquescent. Single-spore isolations showed this species to be heterothallic although most of the ascospores from the parent strain are diploid. Asci from pairing of the mating types predominantly form three or four ascospores.

Ascospores were observed on 5% malt extract and YM agars after 2–4 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 7020 (Billon-Grand 1985).
Mol% G+C: 45.4, CBS 7020 (BD: Kurtzman et al. 1980a).

Origin of the strain studied: Frass from loblolly pine (*Pinus taeda* L.) Mississippi, U.S.A.

Complementary mating types: NRRL YB-1287-82-2 (CBS 7021) and NRRL YB-1287-82-3 (CBS 7022), derived as single-ascospore isolates from the type strain.

Type strain: CBS 7020 (NRRL YB-1287).

Comments: *P. amylophila* is phenotypically quite like *P. mississippiensis* and differs from the latter only in its ability to assimilate soluble starch. Complementary mating types from the two species conjugate but the resulting ascospores are infertile. In a comparison of nDNA relatedness, the two taxa showed only 25% base sequence complementarity (Kurtzman et al. 1980a), but this is still higher than the 0–15% commonly found between more distantly related species. Thus, the data show that these two species have recently evolved from a common ancestor. *P. amylophila* and *P. mississippiensis* are similar to *P. rhodanensis, P. wickerhamii* and *P. veronae,* which are also heterothallic, but Kurtzman et al. (1980a) found that they gave no mating reactions

with these latter three taxa nor did they show significant nDNA relatedness with them.

42.6. *Pichia angophorae* M.W. Miller & Barker (1968)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.0–6.0)×(3.0–7.0) µm, and occur singly, in pairs or in short chains. Growth is butyrous and light tan in color.

Growth on the surface of assimilation media: Thin pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows well-developed pseudohyphae bearing blastoconidia. True hyphae are not formed. Aerobic growth is light tan in color, moderately glistening, butyrous, and with entire margins.

Formation of ascospores: Miller and Barker (1968) reported ascus development in their original isolates to be preceded by iso- or heterogamic conjugations, or rarely by no conjugation. Two to four hat-shaped ascospores are formed in each ascus. Asci are deliquescent. Single-spore isolates yielded both sporogenous and asporogenous cultures and the species was described as both homo- and heterothallic (Miller and Barker 1968). The occurrence of haploid as well as diploid spores could account for these observations. The type strain, NRRL Y-7118 (CBS 5823), is now asporogenous, but conjugates and sporulates when paired with mating type NRRL Y-7843.

Ascospores were observed on 5% malt extract agar after 1–3 days at 25°C, following pairing of complementary mating types.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+/w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 33.0 (J.B. Fiol, cited by Billon-Grand 1981).

Origin of the strains studied: Gum exudate of a red gum tree (*Angophora costata*), Australia (1); mating types derived from the type strain, Kreger-van Rij.

Complementary mating types: NRRL Y-7843 (CBS 5823) and NRRL Y-7844 (CBS 5830), derived from the type strain.

Type strain: CBS 5823 (NRRL Y-7118), from *Angophora costata.*

Comments: *P. angophorae* is phenotypically similar to *P. spartinae* but differs from the latter by its growth on D-xylose and lack of growth on hexadecane, L-sorbose and at 37°C. No response was noted in mixtures of mating types from the two species (Kurtzman and Ahearn 1976).

42.7. *Pichia angusta* (Teunisson, Hall & Wickerham) Kurtzman (1984a)

Synonyms:
Hansenula angusta Wickerham (1951) nom. nud.
Hansenula angusta Teunisson, Hall & Wickerham (1960)
Hansenula polymorpha Falcão de Morais & Dália Maia (1959)
Ogataea polymorpha (Falcão de Morais & Dália Maia) Y. Yamada, Maeda & Mikata (1994c)
Torulopsis methanothermo Urakami (1975) nom. nud.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to elongate, (1.5–4.6)×(1.9–5.3) µm, and single, in pairs, or in small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Very thin pellicles are occasionally produced.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither hyphae nor pseudohyphae are detected under the coverglass. Aerobic growth is white to slightly tannish-white, smooth to finely striated, glistening, and butyrous. Colony margins are entire or finely lobed.

Formation of ascospores: Asci are unconjugated, or exhibit conjugation between parent and bud, or less frequently between independent cells. One to four hemispheroidal or narrow brimmed hat-shaped ascospores are formed per ascus. The asci are deliquescent. Single-spore isolates from four-spored asci from the type strain gave sporogenous colonies, and the species is presumed homothallic (Kurtzman, unpublished).

Ascospores were observed on 5% malt extract, YM, and McClary's acetate agars after 4–7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	w/–	Methanol	+
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	v
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	v
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 7073 and 3 additional strains (Yamada et al. 1973a).
Mol% G + C: 47.8–48.3, CBS 7073 and 3 additional strains (T_m: Nakase and Komagata 1971b); 48.0, CBS 4732 (BD: Kurtzman, unpublished).

Origin of the strains studied: Soil (1); concentrated orange juice (1); *Drosophila* spp. (5); intestinal tract of swine (1); 'alpechin' (1); insect frass from various deciduous trees and nuts (19).

Type strain: CBS 7073 (NRRL Y-2214), isolated from the fruit fly *Drosophila pseudoobscura* in California, U.S.A. (Shehata et al. 1955), and selected by Teunisson et al. (1960) as the type strain of *Hansenula angusta.*

Comments: With the transfer of *Hansenula* species with hat-shaped ascospores to *Pichia* (Kurtzman 1984a), several nomenclatural difficulties arose. One of these concerned *Hansenula polymorpha* because of prior usage of the combination *Pichia polymorpha.* Because of the unavailability of the name *polymorpha, Pichia angusta* was selected for the new combination because *H. angusta* is an obligate synonym of *H. polymorpha.*

Phaff (1985) reported *P. angusta* to be common in rotting opuntia cacti. The cactus isolates show G + C contents about 1 mol% higher than non-cactus strains but reduced (ca. 65–70%) nDNA complementarity with these strains. The cactus isolates differ phenotypically by slow, weak growth on methanol but grow at elevated temperatures (45°–46°C).

42.8. *Pichia anomala* (E.C. Hansen) Kurtzman (1984a)

Anamorph: *Candida pelliculosa* Redaelli
Synonyms:
Saccharomyces anomalus E.C. Hansen (1891)
Willia anomala E.C. Hansen (1904)
Hansenula anomala (E.C. Hansen) H. & P. Sydow (1919)
Endomyces anomalus (E.C. Hansen) Zender (1925b)
Saccharomyces sphaericus von Nägeli (1879)
Hansenula sphaerica (von Nägeli) H. & P. Sydow (1919)
Hansenula anomala (E.C. Hansen) H. & P. Sydow var. *sphaerica* (von Nägeli) Dekker (Stelling-Dekker 1931)
Saccharomyces acetaethylicus Beijerinck (1892)
Monilia javanica Went & Prinsen Geerligs (1894)
Willia javanica Groenewege (1921a)
Hansenula javanica (Groenewege) Dekker (Stelling-Dekker 1931)
Endoblastoderma pulverulentum Fischer & Brebeck (1894)
Mycoderma cerevisiae Desmazières var. *pulverulentum* Beijerinck (cited in Barnett et al. 1990)
Mycoderma pulverulenta (Beijerinck) Nannizzi (1934)
Willia odessa Weber (1922)
Endomyces odessa (Weber) Zender (1925b)
Hansenula odessa (Weber) Dekker (Stelling-Dekker 1931)
Willia schneggii Weber (1922)
Endomyces schneggii (Weber) Zender (1925b)
Hansenula schneggii (Weber) Dekker (Stelling-Dekker 1931)
Hansenula anomala (E.C. Hansen) H. & P. Sydow var. *schneggii* (Weber) Wickerham (1970a)
Willia productiva Berkhout (1923)
Hansenula productiva Beijerinck ex Dekker (Stelling-Dekker 1931)
Hansenula anomala (E.C. Hansen) H. & P. Sydow var. *productiva* Dekker (Stelling-Dekker 1931)
Monilia productiva nom. nud. (cited by Wickerham 1970a)
Endomyces margaritae Zender (1925a)
Willia margaritae (Zender) Guilliermond (1928)
Candida pelliculosa Redaelli (1925)
Mycocandida pelliculosa (Redaelli) Guerra (1935)
Candida javanica (Went & Prinsen Geerligs) Berkhout (1923)
Willia bispora Mattlet (1926)
Hansenula bispora (Mattlet) Nannizzi (1934)
Hansenula anomala (E.C. Hansen) H. & P. Sydow var. *longa* Dekker (Stelling-Dekker 1931)
Hansenula anomala (E.C. Hansen) H. & P. Sydow var. *robusta* Dekker (Stelling-Dekker 1931)
Hansenula nivea Castelli (1933)
Hansenula panis Castelli (1933)
Saccharomyces aceris-sacchari Fabian & Hall (1933)
Hansenula anomala (E.C. Hansen) H. & P. Sydow var. *heteromorpha* Bedford (1942) nom. nud.
Hansenula miso (and forms α, β, β var. 1) Mogi (1942) nom. nud.
Hansenula octospora Mogi (1942) nom. nud.
Hansenula miso var. *octosporus* S. Goto & Yokotsuka (1962b) nom. nud.
Candida pelliculosa Redaelli var. *cylindrica* Diddens & Lodder (1942)
Candida beverwijkii Novák & Vitéz (1964)
Hansenula ukrainica Kvasnikov, Nagornaya & Shchelokova (1979b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to elongate, (1.9–4.1)×(2.1–6.1) µm, and occur singly, in pairs, or in small clusters. Growth is butyrous and faintly tan in color.

Growth on the surface of assimilation media: Pellicles are produced by some strains and when present vary from thin and smooth to thick and folded.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass may show abundant, branched pseudohyphae, although some strains form no pseudohyphae. None of the isolates form true hyphae. Aerobic growth is white to tannish-white and generally butyrous. Some strains are smooth and glistening while others are dull and somewhat chalky. Colony margins range from entire to lobed and occasionally show

a fringe of pseudohyphae. The cultures usually produce a faintly pleasant odor.

Formation of ascospores: *P. anomala* is heterothallic, but the sporogenous diploid form is usually isolated from nature. Diploid cells directly convert to asci and form one to four hat-shaped ascospores (Fig. 130). Asci are deliquescent.

Ascospores were observed on 5% malt extract agar, V8 agar, and sterilized carrot slices after 3–10 days at 25°C.

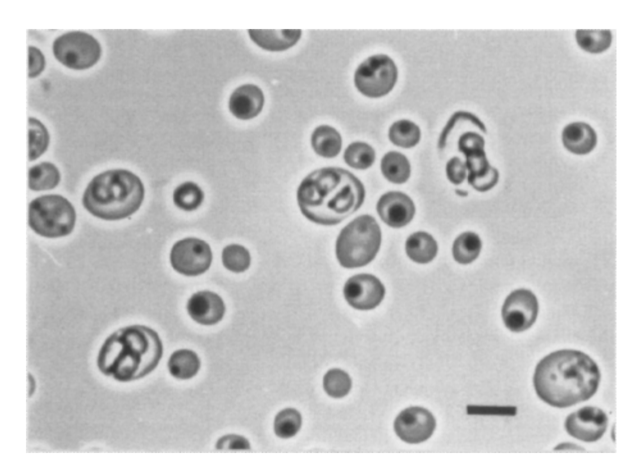


Fig. 130. *P. anomala*, CBS 5759. Ascosporogenous culture after 3 days, 25°C, on 5% malt extract agar. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	w/–
Sucrose	+	Trehalose	–
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	v		

Co-Q: 7, CBS 5759, and 5 additional strains (Yamada et al. 1973a).

Mol% G+C: 35.9–36.6, CBS 5759, AJ 4160, AJ 4167 (T_m: Nakase and Komagata 1971b); 36.8, CBS 605 (T_m: Stenderup and Bak 1968); 35.8–37.4, CBS 5759 and 5 additional strains (BD: Kurtzman 1984b).

Origin of the strains studied: Contaminants from industrial or pilot plant fermentations (12); ragi, Java (10); soil (9); gums of trees, principally *Prunus* (9); stored grain (6); ensilage (4); lakes or streams (3); warm-blooded animals (3); tree exudates (2); frass (2); fermenting mushrooms (2); sewage (2); fruit (1); from various collections and investigators (58).

Complementary mating types: NRRL Y-366-8 (CBS 1984) and NRRL Y-2153-4 (CBS 1982).

Type strain: CBS 5759 (NRRL Y-366), received from Fabian, U.S.A., as *Willia anomala* Saito.

Comments: Wickerham (1970a) separated *Hansenula anomala* var. *schneggii* from *H. anomala* var. *anomala* because the former is less capable of fermenting sucrose than the latter, and it does not grow in osmotic medium. Additionally, the variety *schneggii* does not assimilate raffinose. Perhaps even more striking, the variety *schneggii* produces cylindrical and often markedly elongated thread-like cells while the variety *anomala* does not. However, sexual reactions tend to obscure the other differences between the varieties. Reactions between strains of the variety *schneggii* are weak, but mixtures that contain one strain of each variety are stronger.

Kurtzman (1984b) compared *H. anomala* var. *anomala* and *H. anomala* var. *schneggii* from extent of nDNA reannealing. The two varieties showed 94% base sequence complementarity. Consequently, in view of the great similarity of these strains at the molecular level, the varietal designations have not been maintained. In other nDNA comparisons, *Hansenula ukrainica* was shown to be a synonym of *P. anomala* (Kurtzman 1992b).

42.9. *Pichia antillensis* Starmer, Phaff, Tredick, Miranda & Aberdeen (1984)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, or ellipsoidal to short elongate, (1.5–4.1)×(2.1–7.1) μm, and occur singly, in pairs, or infrequently, in small clusters. Growth is butyrous and light tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass is devoid of hyphae or pseudohyphae, but small outgrowths of undifferentiated cells may form. Aerobic growth is white, glistening, and butyrous with a depressed center and an irregularly lobed margin. A faint musky, acidic odor is present.

Formation of ascospores: This species is heterothallic and is often isolated from nature in the asporogenous haploid state, although diploids are occasionally found. Asci form from naturally occurring diploid cells or those arising following conjugation of complementary mating

types. Asci generally contain four hat-shaped ascospores that are released soon after formation.

Ascospores were observed on YM agar after 5 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G+C: 33.4–33.7, CBS 7111 and 5 additional strains (BD: Starmer et al. 1984).

Origin of the strains studied: Necrotic tissue of the columnar cactus *Cephalocereus royenii* from the Virgin Islands: Prickly Pear Island, Virgin Gorda (CBS 7111, CBS 7113); Beef Island, Tortola (CBS 7112).

Complementary mating types: CBS 7112 (NRRL Y-12882) and CBS 7113 (NRRL Y-12883), isolated by Starmer et al. (1984).

Type strain: CBS 7111 (NRRL Y-12881).

Comments: Starmer et al. (1984) noted that *P. antillensis* shares many physiological and ecological similarities with *P. heedii, P. amethionina, P. opuntiae* and *P. thermotolerans,* all of which are heterothallic. Comparisons of nDNA complementarity showed *P. antillensis* to have, respectively, about 50% and 28% relatedness with *P. opuntiae* and *P. thermotolerans,* but because no conjugation was detected between mating types of the taxa, they are considered separate biological species. At present, *P. opuntiae* is known only from Australia, *P. thermotolerans* from Mexico, and *P. antillensis* from the West Indies. Results of the present comparison are in good agreement with the original description except that one strain (CBS 7113) showed a delayed growth reaction on salicin rather than being unable to assimilate this compound.

42.10. *Pichia barkeri* Phaff, Starmer, Tredick-Kline & Aberdeen (1987a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal to elongate, (2.0–6.0)×(2.5–10.0) μm, and occur singly, in pairs and in small clusters. Growth is butyrous, dull-glistening and tannish-white.

Growth on the surface of assimilation media: Moderate, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant well-branched pseudohyphae with blastoconidia, but no true hyphae. Aerobic growth is a faint yellow-tan in color, butyrous with a dull, roughened surface, and irregularly lobed margins. A faint acidic odor is present.

Formation of ascospores: Ascosporulation begins with the development of a protuberance on a vegetative cell. The terminal end of the protuberance swells and seems to serve as a conjugating cell. Up to four hat-shaped ascospores form in one of the cells, usually the parent cell. Asci become deliquescent soon after spore formation. On the basis of this life cycle, Phaff et al. (1987a) proposed that *P. barkeri* appears to be homothallic.

Ascospores were observed on McClary's acetate agar after 5–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	s
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 7 (Y. Yamada and H.J. Phaff, personal communication).

Mol% G+C: 35.7–36.6, CBS 7256 and 21 additional strains (BD: Phaff et al. 1987a).

Origin of the strains studied: Rotting cladodes of

prickly pear cactus (*Opuntia stricta*) from Discovery Bay, Jamaica (CBS 7256), and Cayman Brac, Caribbean Sea (CBS 7257).

Type strain: CBS 7256 (NRRL Y-17350).

Comments: The work of Phaff et al. (1987a) shows the habitat of *P. barkeri* to be restricted to rotting cladodes of *Opuntia stricta*. Further, *P. barkeri* has been isolated only from *O. stricta* growing on several islands of the Caribbean Sea and from a small area in New South Wales, Australia. *P. barkeri* is phenotypically quite similar to *P. kluyveri* var. *kluyveri* although the two can be separated because *P. barkeri* is characterized by the production of lipase. Additionally, *P. kluyveri* var. *kluyveri* shows killer activity against *Candida glabrata* whereas *P. barkeri* does not (Phaff et al. 1987a). The two taxa show about 20% nDNA complementarity, suggesting that their divergence as separate species has been relatively recent (Phaff et al. 1987a): the G+C content of *P. barkeri* is 6 mol% higher than that of *P. kluyveri*. In contrast to the original description, citrate assimilation was not detected in the present study.

42.11. *Pichia besseyi* Kurtzman & Wickerham (1972)

Synonym:
Yamadazyma besseyi (Kurtzman & Wickerham) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (4.3–7.0)×(4.3–8.2) μm, and occur singly or occasionally in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae, but "tree-like" outgrowths of elongated cells are detected on corn meal agar after 2 weeks. Aerobic growth is dull-glistening and butyrous with an entire margin. The colony color is initially white to cream but becomes pink to brown as ascospores are formed.

Formation of ascospores: Occasionally, asci are formed following conjugation between independent cells, but the majority are unconjugated. Asci usually contain four spores and become deliquescent upon maturity. The shape of ascospores is generally hemispheroidal; for most spores, the underside appears concave and the lower periphery is somewhat thickened, but some spores seem to have a flattened, saturn-like appearance (Fig. 131). Single-spore isolates from four-spored asci are sporogenous and the species is presumed to be homothallic.

Ascospores were observed on 5% malt extract, morphology and YM agars after 1 week at 25°C.

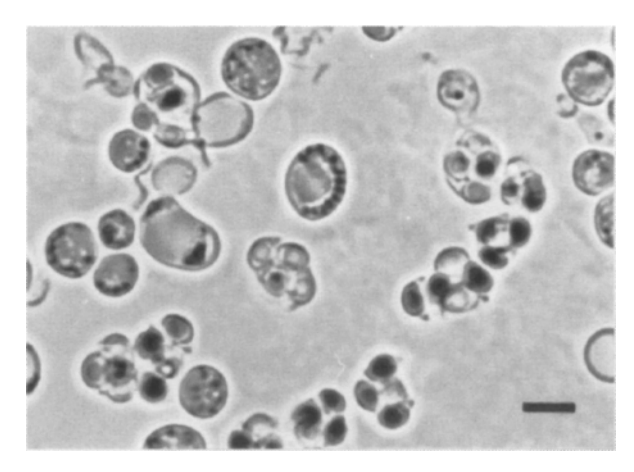


Fig. 131. *P. besseyi*, CBS 6343. Ascosporogenous culture after 3 days, 25°C, on 5% malt extract agar. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	+
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 6343 [Yamada, pers. comm.; the determination of Co-Q 9 by Billon-Grand (1985) appears incorrect].

Mol% G+C: 35.1, 35.2, CBS 6343 (T_m: Billon-Grand 1981; BD: Kurtzman, unpublished).

Origin of the strain studied: Shallow water in a marsh near Bonaventure, Gaspé Peninsula, Quebec, Canada (1).

Type strain: CBS 6343 (NRRL YB-4711).

Comments: *P. besseyi* assimilates only a limited number of carbon compounds making it phenotypically similar to *P. membranifaciens*. However, it assimilates mannitol and glucitol, compounds on which *P. membranifaciens* is incapable of growth. Likewise, *P. besseyi* assimilates lactic acid, but not ribitol and may be distinguished

from *P. delftensis* by these characters. rRNA comparisons place *P. besseyi* in the *Saturnispora* clade (Kurtzman and Robnett, manuscript in preparation).

42.12. *Pichia bimundalis* (Wickerham & Santa María) Kurtzman (1984a)

Synonyms:

Hansenula bimundalis Wickerham & Santa María (Wickerham 1965b)

Candida bimundalis Wickerham & Santa María var. *bimundalis* (Wickerham 1965b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.8–4.3)×(2.0–5.2) µm, and single or in pairs. Growth is butyrous and light cream colored.

Growth on the surface of assimilation media: Usually pellicles are not formed, but occasional strains give waxy climbing pellicles.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass consists of abundant and moderately well differentiated pseudohyphae. True hyphae are also present. Aerobic growth is white to tannish-white, butyrous, and glistening or mat rugose. Colonies may be fringed with hyphae or pseudohyphae. An ester-like odor is produced.

Formation of ascospores: This species is heterothallic and only haploid strains have been isolated from nature. Following conjugation between complementary mating types, zygotes may serve as asci or diploid cells arising from the zygotes become asci. The ascospores are hat-shaped with prominent brims and there are usually four per ascus. Asci are deliquescent.

Ascospores were observed on 5% malt extract agar after 3–10 days at 25°C, following pairing of complementary mating types.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	w/–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 5642 (Yamada et al. 1973a).

Mol% G+C: 40.1, 41.1, CBS 5642 (T_m: Nakase and Komagata 1971b; BD: Fuson et al. 1979); 41.5, 42.1, NRRL YB-2805, CBS 5642 (BD: Kurtzman 1984b).

Origin of the strains studied: Larvae of the beetle *Ergates faber* and larvae of three other beetle genera living principally in stumps of Scotch pine (*Pinus sylvestris* Linn.), Spain (8); frass of a fir tree (*Abies firma* Sieb. & Zucc.), Japan (1).

Complementary mating types: NRRL Y-5343 (CBS 5642) and NRRL Y-5354 (CBS 5643).

Type strain: CBS 5642 (NRRL Y-5343), isolated from a larva of *Ergates faber* (Wickerham 1965b).

Comments: The close relationship between *P. bimundalis* and *P. americana* is discussed in the treatment of the latter species.

42.13. *Pichia bispora* (Wickerham) Kurtzman (1984a)

Synonyms:

Endomyces bisporus Beck (1922)

Endomycopsis bispora (Beck) Dekker (Stelling-Dekker 1931)

Hansenula beckii Wickerham (1951)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate and measure (1.7–5.8)×(2.8–11.3) µm. They occur singly, in pairs, or in small clusters. Growth is light tan in color, and, depending upon the isolate, is either butyrous or mycelial.

Growth on the surface of assimilation media: Pellicles or rings are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and well-branched pseudohyphae as well as true hyphae. Aerobic growth is light tan in color and may be butyrous or mycelial. Colony margins are entire or finely lobed. A faint ester-like odor is present.

Formation of ascospores: Beck (1922) reported the asci to be free cells in which two hat-shaped spores were formed. Subsequent investigators found Beck's strain (CBS 1890) to be asporogenous (Kreger-van Rij 1970c), but her original description leaves no doubt about the presence of ascospores. An additional strain (CBS 6468, NRRL Y-11610) of *P. bispora* became available, and this culture formed two to four hat-shaped spores per ascus. Asci are single, unconjugated cells which become deliquescent upon maturation of the ascospores (Fig. 132). Colonies of single-spore isolates from four-spored asci proved sporogenous and the species is presumed to be homothallic (Kurtzman, unpublished).

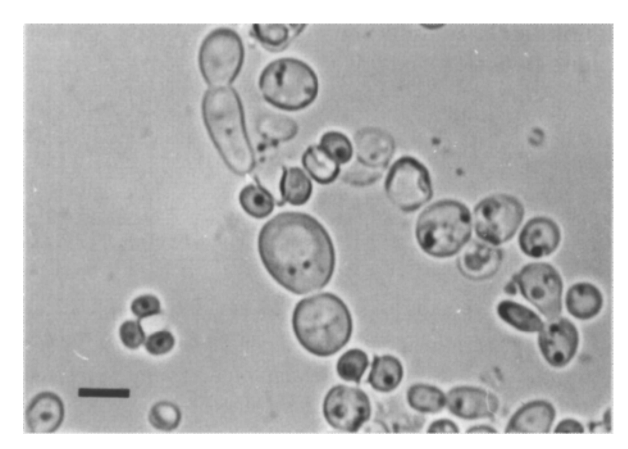


Fig. 132. *P. bispora*, CBS 6468. Ascosporogenous culture after 3 days, 25°C, on YM agar. Bar = 5 μm.

Ascospores were observed on YM agar after 3–5 days at 25°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+/w
Raffinose	–	α-Methyl-D-glucoside	+/w
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	w/–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 1890 (Yamada et al. 1973a).
Mol% G + C: 36.6, 37.1, CBS 1890 (T_m: Nakase and Komagata 1971b; BD: Fuson et al. 1979).

Origin of the strains studied: Frass from tunnels in the bark of a spruce (*Abies* sp.), Beck, Austria (1); human scalp, Laaksonen, Finland (1).

Type strain: CBS 1890 (NRRL Y-1482), frass from spruce bark.

Comments: In their study of septa in ascomycetous yeasts by electron microscopy, Kreger-van Rij and Veenhuis (1973) showed *P. bispora* (*Endomycopsis bispora*) to have hyphal septa with a closure line typical of other mycelial *Pichia* species. In view of this, *P. bispora* has been retained in *Pichia*.

Strain CBS 6468 (NRRL Y-11610) was found to differ from the type strain by giving a weak and late fermentation of glucose and by the inability to assimilate ribitol, mannitol, and lactic acid. In a comparison of their nDNAs, the two strains exhibited 97% base sequence complementarity and represent the same species (Kurtzman, unpublished).

Separation of *P. bispora, P. canadensis, P. americana* and *P. alni* using standard tests is problematical; however, DNA reassociation studies clearly show the species to be genetically isolated (Fuson et al. 1979) despite their many phenotypic similarities. The strong growth shown by *P. canadensis* at 37°C serves to separate it from the other three species, which fail to grow at this temperature. *P. americana* and *P. bispora* form true hyphae whereas *P. alni* does not. Glucose fermentation by *P. americana* is weak or strong and occurs early (1–10 days), in contrast to *P. bispora* which either does not ferment glucose or gives a late (greater than 10 days) and weak fermentation of this sugar.

42.14. *Pichia bovis* van Uden & do Carmo-Sousa (1957)

Synonym:
Zymopichia bovis (van Uden & do Carmo-Sousa) Novák & Zsolt (1961).

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (1.9–5.1) × (2.1–9.2) μm, and occur singly or in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Dry climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth of pseudohyphae under the coverglass may be quite rudimentary or moderately well developed. True hyphae are not formed. Aerobic growth is tannish-white, smooth, faintly glistening, butyrous, and with an entire margin. A faint ester-like odor is present.

Formation of ascospores: Asci are unconjugated and generally contain no more than two spores. The ascospores are hat-shaped and liberated from the asci soon after formation.

Ascospores were observed on 5% malt extract agar after 2–3 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	w/–	Trehalose	v
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 2616 (Yamada and Kondo 1972b).
Mol% G+C: 39.8, 42.3, CBS 2616 (T_m: Nakase and Komagata 1970b; BD: Kurtzman, unpublished).

Origin of the strains studied: Cecum of a cow, van Uden (1); from Santa María (1).

Type strain: CBS 2616 (NRRL YB-4184), from the cecum of a cow.

Comments: *Pichia bovis* is phenotypically quite similar to *P. onychis,* but differs from the latter by the inability to ferment and to assimilate raffinose.

42.15. *Pichia burtonii* Boidin, Pignal, Lehodey, Vey & Abadie (1964)

Anamorph: *Candida variabilis* (Lindner) Berkhout
Synonyms:
Endomycopsis burtonii (Boidin, Pignal, Lehodey, Vey & Abadie) Kreger-van Rij (1970b)
Hyphopichia burtonii (Boidin, Pignal, Lehodey, Vey & Abadie) von Arx & van der Walt (1976)
Monilia variabilis Lindner (1898b)
Oospora variabilis (Lindner) Lindau (1907)
Candida variabilis (Lindner) Berkhout (1923)
Trichosporon variabile (Lindner) Delitsch (Lembke 1943)
Dematium chodati Nechitsch (1904)
Cladosporium chodati (Nechitsch) Saccardo (1906)
Candida chodati (Nechitsch) Berkhout (1923)
Endomycopsis chodati Wickerham & Burton (1952) nom. nud.
?*Sporotrichum carougeaui* Langeron (1922)
Sporotrichum anglicum Castellani (1937b)
Trichosporon behrendii Lodder & Kreger-van Rij (1952)
Fermentotrichon behrendii (Lodder & Kreger-van Rij) Novák & Zsolt (1961)
Candida armeniaca-cornusmas Sarukhanyan (1957)
Candida fibrae Nakase (1971a)
Cladosporium fermentans S. Goto, Yamakawa & Yokotsuka (1975a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2.3–5.7)×(6.0–9.0) µm, and occur singly, in pairs and in small clumps. Pseudohyphae and true hyphae may be present. Growth is tannish-yellow in color and may be butyrous to somewhat mycelial.

Growth on the surface of assimilation media: Depending upon the strain, pellicles may be thin and incomplete or quite thick.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant pseudohyphae as well as true hyphae. Blastoconidia may be borne on denticles. Denticles occur on blastoconidia as well as on hyphae and pseudohyphae. Arthroconidia are also produced. Aerobic growth is white to tannish-white in color, dull and sometimes powdery, and usually fringed by a broad mycelial band. A faint ester-like odor is present.

Formation of ascospores: This species is heterothallic. Asci may form directly from diploid cells or result from the pairing of complementary mating types. Each ascus contains one to four hat-shaped ascospores which are readily released at maturity (Fig. 133).

Ascospores were observed on 5% malt extract- and YM agars after 3–5 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	w/–	Raffinose	+/w
Sucrose	+	Trehalose	s
Maltose	+/w		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+/w	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	+/w
Saccharate	–	Growth at 37°C	w/–
10% NaCl/5% glucose	+		

Co-Q: 8, CBS 2352, AJ 4275 (Yamada et al. 1976b).
Mol% G+C: 35.6, AJ 4832 (T_m: Nakase and Komagata 1971g); 37.3, CBS 2352 (BD: Kurtzman, unpublished).

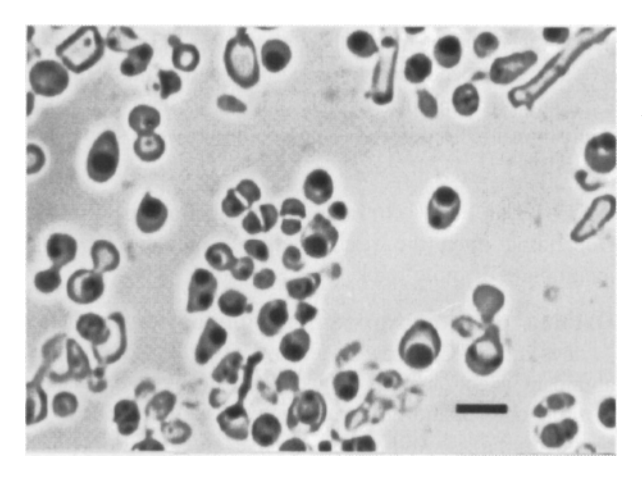


Fig. 133. *P. burtonii*, CBS 2352×CBS 2353. Ascosporogenous culture after 3 days, 25°C, on 5% malt extract agar. Bar = 5 μm.

Origin of the strains studied: Pollen carried by wild bees in Brazil (1); silage (1); stored rice (6); wheat (2); wheat flour (3); chicken feed (7); fig tree (1); wound on palm tree (1); ragi (1); sputum (1); skin (1); other substrates (6).

Complementary mating types: NRRL Y-1933 (CBS 2352) and NRRL Y-1938 (CBS 2353).

Type strain: CBS 2352 (NRRL Y-1933), from pollen.

Comments: The presence of budding cells and arthroconidia as well as sometimes profuse amounts of true hyphae has prompted the assignment of *P. burtonii* to various genera. In 1952, Wickerham and Burton discovered the ascosporic stage of this species and showed it to be heterothallic. They described it as *Endomycopsis chodati* but a Latin diagnosis was not provided. Boidin et al. (1964) validly described the species as *P. burtonii* and in 1970 Kreger-van Rij (1970b) transferred it to *Endomycopsis*. In 1971, van der Walt and Scott (1971b) showed *Endomycopsis* to be an obligate synonym of *Saccharomycopsis* Schiönning and transferred many of the *Endomycopsis* species to *Saccharomycopsis*, but not *P. burtonii*. Later, von Arx and van der Walt (1976) transferred *P. burtonii* to their newly created genus *Hyphopichia*. Cardinal features of this new genus included heterothallism, presence of septate hyphae, asci formed from conjugating yeast cells and denticulate conidiogenous cells. These workers excluded *P. burtonii* from *Pichia* because it formed expanding septate hyphae and conidia borne on denticles and from *Saccharomycopsis* because it produced asci comprised of pairs of conjugating cells.

In the present treatment, *Hyphopichia* is not accepted because the phenotypic characters used for its definition do not distinguish it from several other genera. Features such as heterothallism, presence of septate hyphae and formation of asci from conjugating cells are widespread. Further, although the denticulate conidiogenous cells of *P. burtonii* are quite pronounced, they are not unique to any one taxon and may be found in *Stephanoascus ciferrii*, *Cephaloascus albidus*, *Yarrowia lipolytica*, *P. rhodanensis*, *P. wickerhamii* and the euascomycete *Aureobasidium pullulans*.

42.16. *Pichia cactophila* Starmer, Phaff, Miranda & M.W. Miller (1978a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (1.2–5.0)×(2.0–8.1) μm, and occur singly, in pairs and in short chains. Growth is butyrous and cream-colored.

Growth on the surface of assimilation media: Thin climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only rudimentary pseudohyphae and no true hyphae. Aerobic growth is slightly glistening, smooth, butyrous, tannish-white in color, and with irregular and finely serrate margins. A faint acidic odor is present.

Formation of ascospores: This species has been isolated from nature in the diploid form and cells convert directly to asci, producing two hat-shaped ascospores that are liberated at maturity. Colonies derived from single ascospores are ascosporogenous, but because tetrads are not formed, it is not certain whether the species is homothallic or heterothallic.

Ascospore formation was observed on YM and 5% malt extract agars after 3–7 days at 25°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+/w	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 6926 (Billon-Grand 1985).

Mol% G+C: 36.0-36.5, CBS 6926 and 2 additional strains (BD: Holzschu et al. 1983).

Origin of the strains studied: Cacti (*Stenocereus* spp., *Lemaireocereus pruinosus*) from Mexico (3).

Type strain: CBS 6926 (UCD-FST 76-243A, NRRL Y-10963) from organ pipe cactus (*Stenocereus thurberi*), Baja California, Mexico.

Comments: Results from the present study are in good agreement with the data of Starmer et al. (1978a), except that D-xylose assimilation was found to be late and either weak or strong rather than negative. None of the strains examined fermented glucose, but Starmer et al. (1978a) reported a few isolates of *P. cactophila* to give a slow and weak fermentation of this sugar, consequently, glucose fermentation is listed as either weak or absent.

P. cactophila has been isolated from a wide variety of cacti (Starmer et al. 1978a, 1990), and this lack of cactus habitat specificity contrasts with the more stringent niche requirements of most other cactophilic *Pichia* spp. *P. cactophila* and *P. pseudocactophila* show about 35% nDNA relatedness. For further discussion of these two species, see the Comments sections under *P. pseudocactophila.*

42.17. *Pichia canadensis* (Wickerham) Kurtzman (1984a)

Anamorph: *Candida melinii* Diddens & Lodder

Synonyms:

Hansenula canadensis Wickerham (1951)
Azymohansenula canadensis (Wickerham) Novák & Zsolt (1961)
Candida melinii Diddens & Lodder (1942)
Hansenula wingei Wickerham (1956)
Endomycopsis wingei (Wickerham) Novák & Zsolt (1961)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to elongate, (1.1–4.6)×(2.8–8.0) µm, and single or in pairs. Growth is butyrous and tannish-white.

Growth on the surface of assimilation media: Pellicles are absent or barely visible.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and highly branched pseudohyphae. True hyphae are also present. Aerobic growth is light tan in color, glistening, and butyrous. Margins of colonies are entire to lobed and may be fringed with pseudohyphae. An ester-like odor is present.

Formation of ascospores: Asci of the type strain are unconjugated single cells that contain two or rarely three hat-shaped ascospores. Asci deliquesce to liberate the spores. Single-spore isolates from two-spored asci gave sporogenous colonies. The low frequency of three-spored asci has prevented their analysis for mating types, and it is not certain whether the type strain is homothallic as originally described (Wickerham 1951).

Strains of *Hansenula wingei,* now considered conspecific with *P. canadensis,* may be haploid or diploid when isolated from nature. Asci usually contain four hat-shaped spores and become deliquescent upon maturity. Strains described as *H. wingei* are heterothallic and it is the first fungus in which sexual agglutination was shown (Wickerham 1956).

Ascospores were observed on YM and 5% malt extract agars after 5–10 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+/w	Galactitol	–
Lactose	–	D-Mannitol	+/w
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+/w	Nitrate	v
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 1991 (Yamada et al. 1973a).

Mol% G+C: 40.9, CBS 661 (T_m: Stenderup and Bak 1968); 39.0–40.0, CBS 1992, CBS 2431 (T_m: Nakase and Komagata 1971b); 39.7–40.8, CBS 1992 and 6 additional strains (BD: Fuson et al. 1979).

Origin of the strains studied: Frass from a red pine (*Pinus resinosa* Ait.), Ontario, Canada (1); frass from an eastern white pine (*Pinus strobus* L.), New Hampshire (1); dead conifer, Wyoming (1); from fungi cultured from a live bark beetle obtained from an Engelmann spruce (*Picea engelmannii* Parry), Colorado (2); Engelmann spruce, Wyoming (3); frass from a dead coniferous tree, Quebec, Canada (1).

Complementary mating types: NRRL Y-2340-5 (CBS 2432) and NRRL Y-2340-21 (CBS 2433), derived as single-ascospore isolates from the type strain of the synonym *Hansenula wingei.*

Type strain: CBS 1992 (NRRL Y-1888), isolated by Wickerham from frass of a red pine.

Comments: Wickerham (1956, 1970a) noted the phenotypic similarity of *P. (H.) canadensis* and *P. (H.) wingei* but

distinguished between the two, observing that *P. canadensis* produced no true hyphae and appeared homothallic while *H. wingei* formed true hyphae and was heterothallic. Indeed, *H. wingei* was quite remarkable in that mixtures of opposite mating types showed strong sexual agglutination (Wickerham 1956). Subsequently, this process was studied in detail by various investigators (Brock 1965, Conti and Brock 1965, Crandall and Brock 1968, Herman et al. 1966, Hunt and Carpenter 1963, Taylor and Orton 1968).

Fuson and coworkers (1979) compared the nDNA relatedness of several *Pichia (Hansenula)* species and showed *H. canadensis* and *H. wingei* to share 78% of their nuclear DNA base sequences. On the basis of previously established guidelines (Price et al. 1978), these workers suggested the two taxa to be conspecific. Kurtzman (unpublished), using somewhat different techniques, found the two species to have 76% nDNA complementarity, thus confirming this unexpected relatedness. Because *H. canadensis* was described 5 years earlier than *H. wingei,* the former species epithet has priority of usage.

Extensive single-spore isolations failed to detect heterothallism in *P. canadensis* and, therefore, precluded demonstration of conspecificity with *H. wingei* through mating studies (Kurtzman, unpublished). However, all single-spore isolates were obtained from two-spored asci, and it is entirely possible that these spores were diploid. By comparison, because of the predominance of diploid spores, heterothallism in *Pichia amylophila* nearly escaped detection (Kurtzman et al. 1980a). Regardless of whether *P. canadensis* is homothallic or heterothallic, considering the mating system as a key character for taxonomic separation seems unsound. It has been shown that the mutation of a single locus in *Saccharomyces cerevisiae* (Hicks and Herskowitz 1976) and in *Schizosaccharomyces pombe* (Leupold 1950) will change the mating behavior from heterothallic to homothallic.

During studies of nDNA relatedness among strains of *P. canadensis* (Kurtzman, unpublished), two isolates were included that were phenotypically similar to the others except that they did not utilize nitrate. Both isolates were obtained from the frass of conifers. When reassociated with NRRL Y-1888, NRRL YB-1915 showed 91% complementarity and NRRL YB-1931 gave 95%. These comparisons have provided additional evidence that nitrate utilization is not a species- or genus-specific character and account for the variable nitrate reaction given for *P. canadensis.*

42.18. *Pichia capsulata* (Wickerham) Kurtzman (1984a)

Anamorph: *Candida molischiana* (Zikes) S.A. Meyer & Yarrow, on the basis of phenotypic similarity (see Comments).

Synonyms:
Hansenula capsulata Wickerham (1951)
Kuraishia capsulata (Wickerham) Y. Yamada, Maeda & Mikata (1994c)
Torula molischiana Zikes (1911)
Torulopsis molischiana (Zikes) Lodder (1934)
Cryptococcus molischianus (Zikes) Skinner (1947b)
Candida molischiana (Zikes) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Torulopsis methanophiles Urakami (1975)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.2–5.0)×(2.4–5.8) μm, and single, in pairs, or occasionally in small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Infrequently, thin pellicles may be produced and mucoid rings are occasionally formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass is devoid of pseudohyphae or true hyphae. Aerobic growth is white to tannish-white, glistening, and mucoid. Cultures usually produce a faint pleasant odor.

Formation of ascospores: There are one or two hat-shaped ascospores per ascus (Fig. 134). Conjugation usually precedes sporulation and may be between a cell and its bud or infrequently between independent cells. In the latter case, conjugants commonly are a cell and the bud of another cell. Asci are deliquescent and spores are liberated soon after formation. Single-spore isolates produce sporogenous cultures, but because no more than two spores are formed per ascus, the evidence that this species is homothallic is not conclusive. However, the presence of parent–bud conjugation is suggestive of homothallism.

Ascospores were observed on YM and 5% malt extract agars after 5–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	+
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	v	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	v	Nitrate	+/w
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	v		

Co-Q: 8, CBS 1993, AJ 4173 (Yamada et al. 1973a).

Mol% G+C: 46.8, 47.1, CBS 1993, CBS 5807 (T_m: Nakase and Komagata 1971b); 46.9, CBS 1993 (BD: Kurtzman, unpublished).

Origin of the strains studied: Frass from mainly coniferous trees (53); soil on humus (3); lichen (1); cattle dung (1).

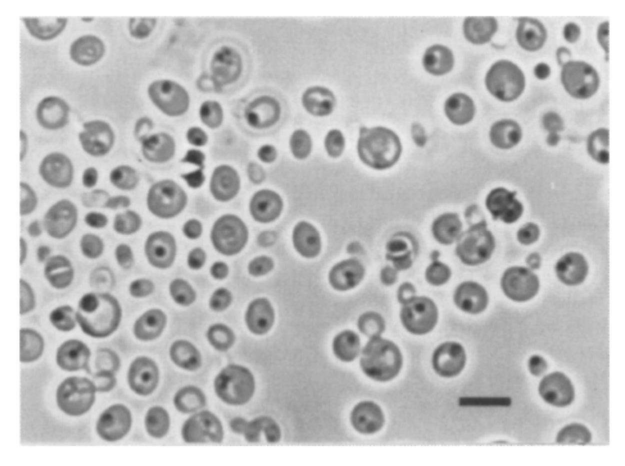


Fig. 134. *P. capsulata,* CBS 1993. Ascosporogenous culture after 3 days, 25°C, on 5% malt extract agar. Bar = 5 µm.

Type strain: CBS 1993 (NRRL Y-1842), isolated by Wickerham from frass of a coniferous tree on the shore of Wabatongushi Lake, near Franz, Ontario, Canada.

Comments: *P. capsulata* produces copious amounts of extracellular polysaccharides. The structures of the mannans and phosphomannans synthesized by this species have been studied in detail by Slodki et al. (1972), Gorin (1973) and Seymour et al. (1976).

Ascosporulation has not been observed for *Candida molischiana,* and the proposed conspecificity with *P. capsulata* is based on similar appearance in culture and the same assimilation profile. However, Lee and Komagata (1983) noted that the two taxa had somewhat different electrophoretic patterns for their cellular enzymes and raised the possibility that they may not represent an anamorph–teleomorph pair.

42.19. *Pichia caribaea* Phaff, Starmer, Lachance, Aberdeen & Tredick-Kline (1992)

Synonym:

Pichia amethionina Starmer, Phaff, Miranda & M.W. Miller var. *fermentans* Starmer & Phaff (1983) nom. nud.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to cylindroidal, (1.8–4.0)×(3.0–10.2) µm, and single, in pairs or in short chains. Growth is butyrous and tannish-white.

Growth on the surface of assimilation media: Pellicles are absent but a film may form.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows some "tree-like" outgrowths comprised of short cells, but well-branched pseudohyphae and true hyphae are lacking. Aerobic growth is butyrous, tannish-white, and glistening with an irregular surface and a finely lobed margin. Colonies are convex in profile. A faint acidic, ester-like odor is present.

Formation of ascospores: This species is heterothallic. Natural isolates may be either haploid or diploid. Diploids, such as the type strain, undergo ascosporulation without prior conjugation and form three to four hat-shaped ascospores that are freed through ascus deliquescence. Paired complementary mating types may show strong sexual agglutination prior to conjugation and ascosporulation.

Ascospore formation occurs on McClary's acetate agar, or less frequently on YM and 5% malt extract agars, after 3–5 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G+C: 34.0–34.4, CBS 7692, and 3 additional strains (BD: Phaff et al. 1992).

Origin of the strains studied: CBS 7692 (UCD-FST 81-62, NRRL Y-17468) from rotting prickly pear cactus (*Opuntia stricta*), Exuma chain, Bahamas; CBS 7695 (UCD-FST 82-570C, NRRL Y-17469) from rotting columnar cactus (*Cephalocereus royenii*), Island of Montserrat, West Indies; CBS 7696 (UCD-FST 83-711.2, NRRL Y-17472) from prickly pear cactus, Conception Island, Bahamas.

Complementary mating types: CBS 7693 [UCD-FST 81-280, NRRL Y-17470 (h^-)] and CBS 7694 [UCD-FST 81-279, NRRL Y-17471 (h^+)], single-spore isolates from UCD-FST 81-36, from the Exhumas chain, Bahamas (Phaff et al. 1992).

Type strain: CBS 7692.

Comments: Phaff et al. (1992) reported ca. 40% nDNA relatedness between *P. caribaea* and each of the two varieties of *P. amethionina.* Interspecific matings gave zygotes, but asci were aberrant and produced malformed ascospores. These data showed the two taxa to be reproductively isolated sibling species. The host range of *P. caribaea* and its geographical distribution throughout the Caribbean was well documented by Phaff et al. (1992).

Results from the present study are in agreement with Phaff et al. (1992) except that NRRL Y-17469 latently assimilated D-xylose in contrast to the reported inability to grow on this pentose, and the strain also failed to grow at 37°C. Phaff et al. (1992) reported that, as for *P. amethionina, P. caribaea* is a naturally occurring auxotroph that requires an organic form of sulfur, such as L-methionine or L-cysteine.

42.20. *Pichia castillae* Santa María & García Aser (1970)

Synonym:

Yamadazyma castillae (Santa María & García Aser) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, (1.4–3.5)×(2.0–7.0) µm, and occur singly, in pairs and in clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin, slightly climbing pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only sparse and poorly differentiated pseudohyphae. True hyphae are not present. Aerobic growth is tannish-white in color, somewhat butyrous, and with a smooth, dull to faintly glistening surface. The margin is lobate. A faint acidic odor is present.

Formation of ascospores: The asci, which show isogamic or heterogamic conjugation, produce two to four hat-shaped ascospores. Spores are released rapidly following their formation. Santa María and García Aser (1970) reported this species to be homothallic.

Ascospores were observed on YM and 5% malt extract agars after 10 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	w
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 6053 (Billon-Grand 1985).

Mol% G+C: 39.5, CBS 6053 (T_m: Billon-Grand 1981).

Origin of the strain studied: Frass of insects infesting the tree *Gymnocladus canadensis* (1).

Type strain: CBS 6053 (NRRL Y-7501).

42.21. *Pichia chambardii* (C. Ramírez & Boidin) Phaff (1956)

Synonyms:

Saccharomyces chambardi C. Ramírez & Boidin (1954) (also in Ramírez & Boidin 1955)

Petasospora chambardi (C. Ramírez & Boidin) Boidin & Abadie (1954)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (1.8–4.1)×(2.6–6.8) µm, and occur singly or in pairs. Growth is butyrous and grayish-white in color.

Growth on the surface of assimilation media: Thin pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only infrequent undifferentiated pseudohyphal strands. True hyphae are not formed. Aerobic growth is white in color, butyrous, smooth, dull-glistening, and with an occasionally lobed margin. A faint acidic odor is present.

Formation of ascospores: Asci are unconjugated and form one to four (usually two) hat-shaped ascospores. The spores are liberated at maturity. It is not known whether the species is homothallic or heterothallic.

Ascospores were observed on 5% malt extract agar after 3 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 1900, IFO 1029, IBL 2575 (Yamada et al. 1973a).
Mol% G+C: 30.2, IFO 1029 (T_m: Nakase and Komagata 1970b); 33.8, CBS 1900 (BD: Kurtzman, unpublished).

Origin of the strain studied: Tanning liquor, France (1).

Type strain: CBS 1900 (NRRL Y-2378).

42.22. *Pichia ciferrii* (Lodder) Kurtzman (1984a)

Synonyms:
Hansenula ciferrii Lodder (1932)
Hansenula anomala (Hansen) H. & P. Sydow var. *ciferrii* Lodder & Kreger-van Rij (1952)
Endomycopsis ciferrii (Lodder) Novák & Zsolt (1961)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (3.3–5.0)×(3.9–9.5) µm, and single, in pairs, or in small clusters. Growth is butyrous and white in color.

Growth on the surface of assimilation media: Pellicles are thin, smooth, and waxy.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and highly branched pseudohyphae. Mycelial variants derived from the type strain, such as NRRL Y-1322, form abundant true hyphae. Aerobic growth is white, faintly glistening to dull, and butyrous. Colony margins are usually lobed and may be fringed with pseudohyphae. A faint ester-like odor is present.

Formation of ascospores: Diploid cells convert to asci and form one to four hat-shaped ascospores. Asci are deliquescent. This species is heterothallic, and Wickerham and Burton (1954b) obtained mating types by heat-treating the sporogenous diploid type culture.

Ascospores were observed on 5% malt extract agar and sterilized carrot slices after 4–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/w	Raffinose	w
Sucrose	+	Trehalose	–
Maltose	v		

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+/w	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+/w	Succinate	+
L-Arabinose	+/w	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+/w	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	+
Saccharate	–	Growth at 37°C	w/–
10% NaCl/5% glucose	w		

Co-Q: 7, CBS 111 (Yamada et al. 1973a).
Mol% G+C: 32.2, CBS 111 (T_m: Nakase and Komagata 1971b).

Origin of the strain studied: Pod from the Tonka-bean tree (*Dipteryx odorata*), Dominican Republic (1).

Complementary mating types: NRRL Y-1031-11 (CBS 1990) and NRRL Y-1031-27 (CBS 1991).

Type strain: CBS 111 (NRRL Y-1031), isolated by Ciferri from Tonka-bean.

Comments: On standard tests, *P. ciferrii* differs from *P. anomala* only in the ability to assimilate rhamnose. Wickerham (1970a) considered the species distinct, however, because they showed no mating response following mixture of their mating types, and *P. ciferrii* produces crystals of tetraacetylphytosphingosine (Wickerham and Stodola 1960, Stodola and Wickerham 1960, Wickerham and Burton 1962), but *P. anomala* has never been observed to produce this compound. Additionally, Nakase and Komagata (1971b) reported *P. ciferrii* to have a G+C content nearly 4% lower than that of *P. anomala* which suggests an almost complete lack of nDNA base sequence complementarity.

42.23. *Pichia delftensis* Beech (1965)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (2.5–5.3)×(2.7–8.9) µm, and occur singly or in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only an occasional undifferentiated pseudohyphal outgrowth. True hyphae are not formed. Aerobic growth is white in color, dull to slightly glistening, butyrous and with a finely lobed margin.

Formation of ascospores: Asci may be unconjugated, or show conjugation between independent cells. One to four hat-shaped ascospores are formed per ascus, and the asci slowly deliquesce. It is not known whether the species is homothallic or heterothallic.

Ascospores were observed on YM and 5% malt extract agars after 3–10 days at 25°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 2614 (Billon-Grand 1985).
Mol% G + C: 33.2, CBS 2614 (BD: Kurtzman, unpublished).

Origin of the strain studied: Naturally fermented apple juice, England (1).

Type strain: CBS 2614 (NRRL Y-7119).

Comments: As noted earlier, *P. delftensis* resembles *P. amethionina* and many other species isolated from cacti.

42.24. *Pichia deserticola* Phaff, Starmer, Tredick & Miranda (1985)

Anamorph: *Candida deserticola* Phaff, Starmer, Tredick & Miranda
Synonym:
Candida deserticola Phaff, Starmer, Tredick & Miranda (1985)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ellipsoidal to elongate, (2.2–4.5) × (2.7–7.6) μm, and single, in pairs and in small clusters. Growth is tannish-white, semi-glistening and butyrous.

Growth on the surface of assimilation media: Thin pellicles often form.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows pseudohyphae that range from poorly differentiated to moderately branched. True hyphae are not produced. Aerobic growth is tannish-white, semi-glistening, butyrous, and convex in profile with a depressed center. Colony margins are smooth to finely scalloped. A faint acidic odor is present.

Formation of ascospores: Asci are often unconjugated but some appear to have undergone conjugation with a bud. Ascospores are hat-shaped and two are formed per deliquescent ascus (Fig. 135). Single-spore isolates form ascosporogenous colonies.

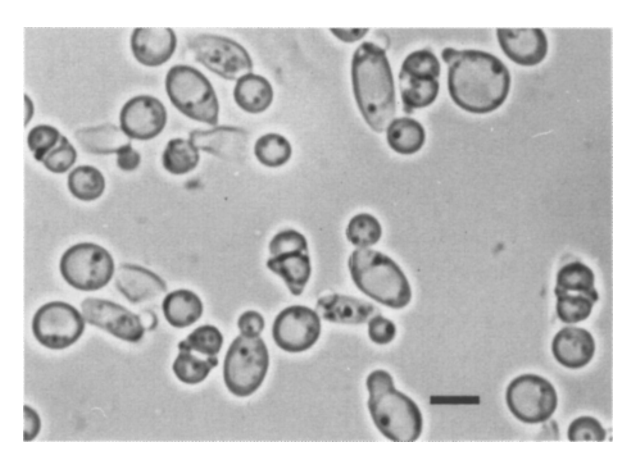


Fig. 135. *P. deserticola*, CBS 7119. Ascosporogenous culture after 10 days, 25°C, on McClary's acetate agar. Bar = 5 μm.

Ascospores were observed on McClary's acetate agar after 2–10 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+/w
D-Xylose	–	Succinate	w
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G + C: 27.4–28.4, CBS 7119 and 7 additional strains (BD: Phaff et al. 1985).

Origin of the strains studied: *P. deserticola*: CBS 7119 (UCD-FST 83-467.3, NRRL Y-12918), from prickly pear cactus (*Opuntia phaeacantha*), Santa Catalina Mountains, Arizona, U.S.A.; CBS 7120 (UCD-FST 81-403B, NRRL Y-12919), from *O. phaeacantha*, Tucson Mountains, Arizona. *Candida deserticola*: CBS 7121 (UCD-FST 76-355A, NRRL Y-12920), type strain, from columnar cactus (*Stenocereus gummosus*), Mulegé, Baja California, Mexico; CBS 7122 (UCD-FST 82-451A, NRRL Y-12921), from columnar cactus (*Stenocereus hystrix*), Gonnaïves, Haiti.

Type strain: CBS 7119.

Comments: Results from the present study are in good agreement with the original description. Phaff et al. (1985) showed nearly 100% nDNA relatedness between *P. deserticola* and the nonascosporogenous strains designated as *Candida deserticola*. The former taxon occurs in species of prickly pear cacti whereas the latter is almost exclusive to columnar cacti. *P. deserticola* and its anamorph are noteworthy because they assimilate so few carbon compounds, although their strong growth on ethyl acetate provides one means to separate them from *P. antillensis*. It should be pointed out that *P. deserticola* has the lowest nDNA base composition of any known yeast.

42.25. *Pichia dryadoides* (D.B. Scott & van der Walt) Kurtzman (1984a)

Synonym:
Hansenula dryadoides D.B. Scott & van der Walt (1971a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal or ellipsoidal, (2.5–5.3) × (2.6–7.7) µm, and single, in pairs, and in small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed but occasional incomplete rings may be observed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only rudimentary pseudohyphae. True hyphae are not produced. Aerobic growth is tannish-white, glistening, and butyrous. Margins vary from entire to lobed. A faintly acidic odor is present.

Formation of ascospores: Diploid cells convert directly to asci and each ascus contains two, or less frequently one, hat-shaped ascospores with prominent brims. Asci are persistent. Scott and van der Walt (1971a) reported that heat treatment of sporogenous cultures gave colonies which sporulated, thus suggesting this species to be homothallic. However, ascospores from two-spored asci may be diploid.

Ascospores were observed on 5% malt extract and YM agars after 3–10 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G + C: 31.8, 31.0, CBS 6154 (HPLC: Miller and Baharaeen 1979; BD: Phaff et al. 1983).

Origin of the strains studied: From tunnels of the pin hole borer *Platypus externedentatus* Fairm. infesting various figs (*Ficus* spp.), Natal, South Africa (2).

Type strain: CBS 6154 (NRRL Y-10990), isolated from *Ficus sycomorus* L.

Comments: Minor differences in growth reactions were noted between the original description and the present study. Scott and van der Walt (1971a) reported growth on D-xylose, salicin, in osmotic medium and at 37°C. Of the two strains examined in the present study, neither grew on salicin nor in osmotic medium, and one failed to grow on D-xylose as well as at 37°C. Scott and van der Walt (1971a) noted that *Candida berthetii* is rather similar to *P. dryadoides*, but the 5% difference in G + C that Poncet (1973a) detected between the two taxa would preclude conspecificity.

42.26. *Pichia euphorbiae* van der Walt & Opperman (1983)

Anamorph: *Candida euphorbiae* van der Walt & Opperman

Synonym:
Candida euphorbiae van der Walt & Opperman (1983)

Growth on 5% malt extract agar: After 3 days at

25°C, the cells are occasionally spheroidal, but usually ellipsoidal to cylindroidal, (1.1–6.0)×(2.2–12.1) µm, and single, in pairs, and in small clusters. Growth is butyrous, slightly glistening and tannish-white.

Growth on the surface of assimilation media: Pellicles were not observed, but rings and islets of surface growth occur.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass is comprised of well-branched pseudohyphae with blastoconidia; true hyphae are present as well. Aerobic growth is white to tannish-white, moderately glistening and butyrous. Colony margins are scalloped with hyphal outgrowths. A faintly acidic odor is present.

Formation of ascospores: This species is heterothallic and has been isolated from nature only as haploid mating types. Cultures of paired complementary mating types form asci with one to four hat-shaped ascospores. Asci are deliquescent.

Ascospores were observed on 5% malt extract agar after 10–15 days at 15°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	ws	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°	v
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G + C: Not determined.

Origin of the strains studied: Insect infested spurge (*Euphorbia ingens* E. Mey.), Groblersdal area, South Africa (2).

Complementary mating types: CBS 7082 (NRRL Y-17233) and CBS 7083 (NRRL Y-17757).

Type strain: CBS 8033 (NRRL Y-17232), a diploid constructed by the mating of CBS 7082 and CBS 7083.

Comments: Van der Walt and Opperman (1983) noted *P. euphorbiae* to be phenotypically similar to *P. rhodanensis, P. wickerhamii, P. amylophila, P. mississippiensis, P. veronae* and *P. meyerae,* which are also heterothallic. Pairings with these species gave a mating reaction only with *P. meyerae.* The reaction was limited to formation of infrequent zygotes that failed to produce ascospores, suggesting the two taxa to be closely related but reproductively isolated species.

42.27. *Pichia euphorbiiphila* (van der Walt) Kurtzman (1984a)

Anamorph: *Candida euphorbiiphila* van der Walt

Synonyms:

Hansenula euphorbiiphila (as *H. euphorbiaphila*) van der Walt (1982b)

Candida euphorbiiphila (as *C. euphorbiaphila*) van der Walt (1982b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are short ellipsoidal to cylindroidal, (2.0–4.6)×(2.3–8.5) µm, and occasionally tapered. Growth is butyrous, glistening and white to tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed but rings are occasionally seen.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, well-branched pseudohyphae with blastoconidia. True hyphae are not formed. Aerobic growth is white to tannish-white, glistening, butyrous, convex in profile with a slight central depression, and with margins that are smooth to finely scalloped. There is a faint odor of esters.

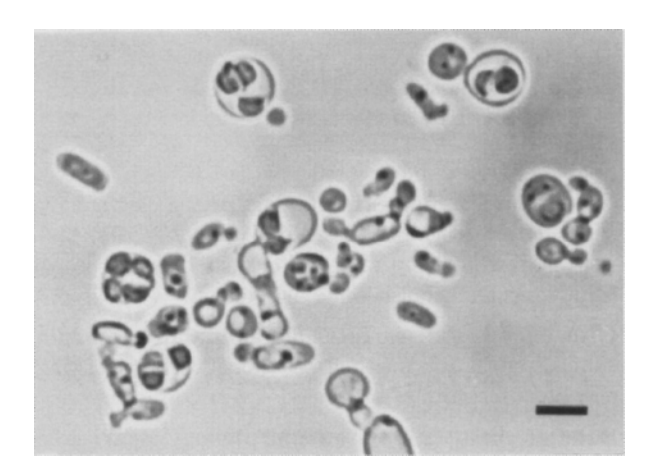


Fig. 136. *P. euphorbiiphila,* CBS 8083. Ascosporogenous culture after 3 days, 25°C, on McClary's acetate agar. Bar = 5 µm.

Formation of ascospores: This species is heterothallic but has been isolated from nature only in the diploid state. Asci from diploid strains are unconjugated and produce two to four hat-shaped ascospores (Fig. 136). Asci become deliquescent at maturity. Van der Walt (1982b) isolated

haploid mating types from the sporulated type strain through heat treatment.

Ascospores were observed on McClary's acetate agar, and to a lesser extent on 5% malt extract agar, after 2–5 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°	+
10% NaCl/5% glucose	w/–		

Co-Q: Not determined.
Mol% G + C: 47.3, CBS 8083 (BD: Kurtzman, unpublished).

Origin of the strains studied: CBS 8083 (NRRL Y-12742), from insect infested decaying tissue of spurge (*Euphorbia ingens*), Transvaal, South Africa; CBS 7912 (NRRL Y-12743) and CBS 7913 (Y-12744), which were derived from heat-treatment of the type strain.

Complementary mating types: CBS 7912 (*a*) and CBS 7913 (*α*), derived from CBS 8083.

Type strain: CBS 8083.

Comments: Van der Walt (1982b) noted the phenotypic similarity of *P. euphorbiiphila* with *P. americana* and *P. bimundalis,* but mating types of *P. euphorbiiphila* showed no response to those of the latter two species.

42.28. *Pichia fabianii* (Wickerham) Kurtzman (1984a)

Anamorph: *Candida fabianii* K. Kodama, Kyono, Iida & Onoyama
Synonyms:
Hansenula fabianii Wickerham (1965a)
Candida fabianii K. Kodama, Kyono, Iida & Onoyama (1964a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.0–4.2)×(2.0–6.5) μm, and single, in pairs, or in small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin pellicles and rings are produced.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional pseudohyphae. True hyphae are not produced. Aerobic growth is tannish-white, glistening, and butyrous. Margins are entire or lobed; pseudohyphae are not formed around the periphery of aerobic colonies. An ester-like odor is produced.

Formation of ascospores: This species is heterothallic and only asporogenous haploid isolates have been obtained from nature. Following the mixture of complementary mating types, zygotes may directly convert to asci or they may bud diploid cells which can become asci. The asci contain one to four hat-shaped ascospores and become deliquescent upon maturity.

Ascospores were observed on 5% malt extract agar after 5–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w
Sucrose	+	Trehalose	–
Maltose	ws		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°	+
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 5640 and 3 additional strains (Yamada et al. 1973a).
Mol% G + C: 44.4–45.6, CBS 5640 and 5 additional strains (T_m: Nakase and Komagata 1971b); 46.7, CBS 5640 (BD: Kurtzman, unpublished).

Origin of the strains studied: A contaminant of a fermentor inoculated with *Aerobacter aerogenes* for production of butylene glycol, U.S.A. (1); alcohol fermentation plant, Cheju Island, Korea (3); rice koji, Japan (4).

Complementary mating types: NRRL Y-1871 (CBS 5640) and NRRL Y-1873 (CBS 5641).

Type strain: CBS 5640 (NRRL Y-1871), isolated from a fermentor used for butylene glycol production.

Comments: Of the heterothallic species of *Pichia, P. fabianii* is most like *P. subpelliculosa*. The two are dissimilar only in their assimilation of erythritol, which is negative for *P. fabianii*. Nakase and Komagata (1971b) reported the G+C contents of the two species to differ by nearly 11% and this would preclude their conspecificity.

42.29. *Pichia farinosa* (Lindner) E.C. Hansen (1904)

Synonyms:
Saccharomyces farinosus Lindner (1894)
Zygosaccharomyces farinosus (Lindner) Papadakis (1922)
Zygopichia farinosa (Lindner) Klöcker (1924)
Yamadazyma farinosa (Lindner) Billon-Grand (1989)
Pichia miso Mogi (1939) nom. nud.
Saccharomyces miso (Mogi) Novák & Zsolt (1961) nom. nud.
Zygopichia miso Mogi (1942) nom. nud.
Zygosaccharomyces tikumaensis Mogi (1942) nom. nud.
Pichia mogii Ohara & Nonomura (1954a) nom. nud.
Zygopichia sake Naganishi (1941a) nom. nud.
Pichia sake (Naganishi) Ohara & Nonomura (1954b) nom. nud.
Zygopichia farinosa (Lindner) Klöcker var. *japonica* Naganishi (1941b) nom. nud.
Pichia farinosa (Lindner) E.C. Hansen var. *japonica* Ohara & Nonomura (1954b) nom. nud.
Pichia minuscula Soneda (1959)
Debaryomyces halotolerans Sasaki & Yoshida (1966) nom. nud.
Candida cacaoi H.R. Buckley & van Uden (1968)
Pichia petrophilum Mu (Mu et al. 1979)
Pichia sorbitophila Rodrigues de Miranda, Appel & Seyfarth (1980)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (1.5–5.0)×(3.0–18.0) μm, and occur singly, in pairs and in short chains. Growth is white to occasionally faintly yellowish-white in color.

Growth on the surface of assimilation media: Heavy, dry climbing pellicles develop.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows pseudohyphae which, depending upon the strain, may be poorly differentiated to moderately branched. True hyphae are not formed. Aerobic growth is white, smooth to rugose, powdery, and generally has lobed margins. A faint ester-like odor is produced by some strains.

Formation of ascospores: Asci show parent cell–bud conjugation and produce one to four spheroidal to subspheroidal ascospores (Fig. 137). Asci are generally persistent, although free spores are occasionally observed. Single-spore isolates from four-spored asci from the type strain and one additional strain were sporogenous, indicating the species to be homothallic (Kurtzman, unpublished). Kawakami et al. (1961) and Kreger-van Rij (1970c) reported the spores to have

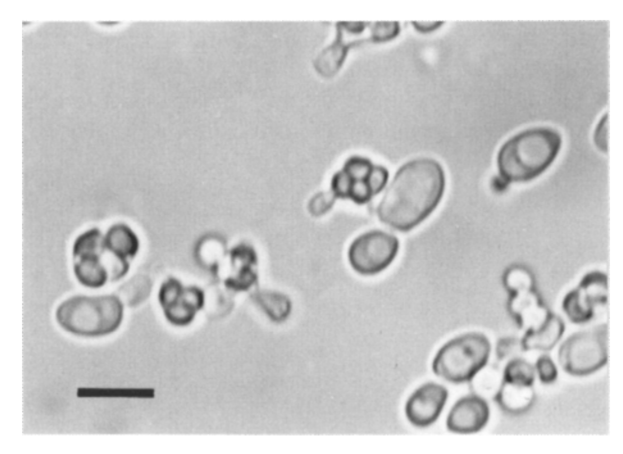


Fig. 137. *P. farinosa*, NRRL Y-2060. Ascosporogenous culture after 3 days, 25°C, on V8 agar. Bar = 5 μm.

a thin, subequatorial ledge visible under the electron microscope.

Ascospores were observed on V8, RG and YM agars after 3–5 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	w/–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	v	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	–
D-Xylose	v	Succinate	v
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°	+
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 185 and 5 additional strains (Yamada et al. 1973a).
Mol% G+C: 38.8–39.8, AJ 4134, AJ 4136, AJ 4138 (T_m: Nakase and Komagata 1970b); 42.7, CBS 185 (BD: Kurtzman, unpublished).

Origin of the strains studied: 'Jopenbier' (1); fermenting cacao, Trinidad (1); miso (2); stored rice (4); wheat flour (1); other substrates (2); *Debaryomyces*

halotolerans (2); *P. sorbitophila* (CBS 7064, NRRL Y-12695); *P. petrophilum* (CBS 7911, NRRL Y-11953).

Type strain: CBS 185 (NRRL Y-7553) from 'Jopenbier'.

Comments: The extent of nDNA relatedness between the type strain of *P. farinosa* and the type strains of *Debaryomyces halotolerans, P. sorbitophila* and *P. petrophilum* was 100%, 70% and 72%, respectively (Kurtzman, unpublished). These results indicate the latter three taxa to be conspecific with *P. farinosa.*

42.30. *Pichia fermentans* Lodder (1932)

Anamorph: *Candida lambica* (Lindner & Genoud) van Uden & H.R. Buckley, on the basis of phenotypic similarity.

Synonyms:
Zymopichia fermentans (Lodder) Novák & Zsolt (1961)
Saccharomyces pastorianus-arborescens van Laer (1902)
Mycoderma lambica Lindner & Genoud (1913)
Candida lambica (Lindner & Genoud) van Uden & H.R. Buckley (1970)
Torula monosa Kluyver (1914)
Mycotorula monosa (Kluyver) Harrison (1928)
Candida monosa (Kluyver) Diddens & Lodder (1942)
Saccharomyces dombrowskii Sacchetti (1933)
Pichia dombrowskii Sacchetti, nom. nud.? (Lodder & Kreger-van Rij 1952)
Saccharomyces scandinavicus Dietrichson (1954)
Candida fimetaria Soneda (1959)
Candida krusei (Castellani) Berkhout var. *transitoria* Saëz (1965)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, (1.9–6.5)×(4.0–14.4) µm, and occur singly, in pairs and in short chains. Growth is tannish-yellow in color.

Growth on the surface of assimilation media: Thin to thick climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and moderately branched pseudohyphae. True hyphae are not present. Aerobic growth is white and dull with the surface wrinkled by convoluted growth. Margins are lobate. A weak ester-like odor is produced by some strains.

Formation of ascospores: Asci may be unconjugated or show conjugation between independent cells or between a parent cell and a bud. Two to four ascospores are formed per ascus, and asci become deliquescent. Spores are hat-shaped but the ledge is not always easily detected under the light microscope. Single-spore isolates from four-spored asci are ascosporogenous, indicating the species to be homothallic.

Ascospores were observed on YM, 5% malt extract, and V8 agars after 10–20 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°	+
10% NaCl/5% glucose	v		

Co-Q: 7, CBS 246 (Yamada et al. 1973a).

Mol% G+C: 42.2, AJ 4458, AJ 4891 (T_m: Nakase and Komagata 1970b); 43.1, CBS 187 (BD: Fuson et al. 1980).

Origin of the strains studied: Buttermilk (1); stracchino cheese (1); sputum (1); spoiled orange juice (1); kefyr (1); cecum of cow (1); *Cyanochen cyanopterus* (1); *Cygnus olor* (1).

Type strain: CBS 187 (NRRL Y-1619) from buttermilk.

Comments: There is considerable phenotypic similarity between *P. fermentans* and *P. kluyveri* var. *kluyveri.* However, the two may be separated on the basis of the strong assimilation of citrate and D-xylose shown by *P. fermentans* (De Camargo and Phaff 1957). These species also differ by about 14% in G+C contents.

42.31. *Pichia finlandica* Kurtzman (1984a)

Synonyms:
Hansenula wickerhamii Capriotti (1961c)
Ogataea wickerhamii (Capriotti) Y. Yamada, Matsuda, Maeda & Mikata (1995a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal or ellipsoidal, (1.4–3.3)×(1.4–3.5) µm, and single or in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not produced.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither hyphae nor pseudohyphae are detected under the coverglass. Aerobic growth is tannish-white, smooth, glistening, and butyrous. Colony margins are entire to finely lobed.

Formation of ascospores: Asci generally form after conjugation between independent cells, but occasionally

they are unconjugated or a parent cell may conjugate with a bud. The asci usually produce three or four rather small hat-shaped ascospores that are soon released by deliquescence. Single-spore isolates give ascosporogenous colonies and the species appears to be homothallic (Kurtzman, unpublished).

Ascospores were observed on 5% malt extract agar after 2–3 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	w/–
L-Arabinose	v	Citrate	–
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 4307 (Yamada et al. 1973a).
Mol% G + C: 45.4, CBS 4307 (T_m: Nakase and Komagata 1971b).

Origin of the strains studied: Soil from a swamp in which spruce and broad-leafed trees were growing, Hÿÿttiala Province of Tavastia Australis, Finland (2).

Type strain: CBS 4307 (NRRL YB-4943), Capriotti's strain 241-A which he had designated as the type of *Hansenula wickerhamii*.

Comments: With the transfer of species of *Hansenula* with hat-shaped ascospores to *Pichia* (Kurtzman 1984a), the name *Hansenula wickerhamii* was replaced owing to earlier usage of the validly described binomial *Pichia wickerhamii* (van der Walt) Kreger-van Rij. The species epithet *finlandica* was chosen to denote the country from which the species was isolated.

42.32. *Pichia fluxuum* (Phaff & Knapp) Kreger-van Rij (1964b)

Synonyms:
Debaryomyces fluxuum (as *D. fluxorum*) Phaff & Knapp (1956)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2.0–6.1)×(3.5–10.5) µm, and occur singly or in pairs. Growth is yellowish-white in color.

Growth on the surface of assimilation media: Dry climbing pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional, sparingly branched pseudohyphae but no true hyphae. Aerobic growth is tannish-white, dull, smooth to partly wrinkled, and with an entire to broadly lobed margin.

Formation of ascospores: Asci are unconjugated and form one or two, rarely three or four, spheroidal, roughened spores (Fig. 138). In addition to the roughening, electron microscopy reveals the spores to have a thin subequatorial ring (Kawakami and Nehira 1958, Kreger-van Rij 1964b, Kurtzman and Smiley 1974). Asci are persistent. It is unknown whether the species is homothallic or heterothallic.

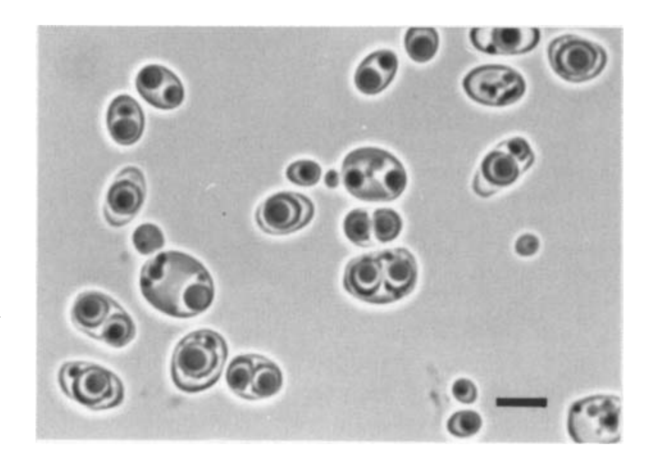


Fig. 138. *P. fluxuum*, CBS 2287. Ascosporogenous culture after 8 days, 15°C, on 5% malt extract agar. Bar = 5 µm.

Ascospores were observed on YM, 5% malt extract and V8 agars after 3–10 days at 15 or 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 2287 (Yamada et al. 1973a).

Mol% G+C: 32.2, 33.8, CBS 2287 (T_m: Nakase and Komagata 1970b; BD: Price et al. 1978).

Origin of the strains studied: Slime flux of oak (*Quercus kelloggii*) (2); slime flux of fir (*Abies concolor*) (2); sauerkraut (3).

Type strain: CBS 2287 (NRRL YB-4273), from *Quercus kelloggii.*

42.33. *Pichia galeiformis* Endo & S. Goto (Goto et al. 1987b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to cylindroidal, (2.6–3.7)×(3.6–7.5) μm, and single, in pairs and small clusters. Growth is tannish-white, dull and butyrous.

Growth on the surface of assimilation media: Moderately heavy climbing pellicles are produced.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows well-branched pseudohyphae with blastoconidia, but true hyphae are not formed. Aerobic growth is tannish-white, dull and butyrous. Colonies are convex with finely scalloped margins. A faint musky odor is present.

Formation of ascospores: Vegetative cells are directly transformed into unconjugated asci which produce 1–4 hat-shaped ascospores. Asci deliquesce on maturity. It is not known whether this species is homothallic or heterothallic.

Ascospores were formed on McClary's acetate agar after 5 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	w
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 7324 (Goto et al. 1987b).

Mol% G+C: 35.6, CBS 7324 (T_m: Goto et al. 1987b).

Origin of the strains studied: CBS 7324 (NRRL Y-17349), from feces, Japan; CBS 763 (NRRL Y-17348), originally from Guilliermond.

Type strain: CBS 7324.

Comments: Assimilation reactions determined in the present study differ considerably from those listed in the original description of this species. Goto et al. (1987b) reported assimilation of sucrose, maltose, cellobiose, raffinose, melezitose and soluble starch (weak), but absence of growth on glycerol, lactate and succinate. Comparisons of large subunit rRNA partial sequences suggest *P. galeiformis* to be a sibling species of *P. membranifaciens* (Kurtzman and Robnett, manuscript in preparation). Separation of these two species is discussed in the Comments section of *P. membranifaciens.*

Note in proof: Rodrigues et al. (1996) reported high nDNA relatedness between *P. galeiformis* and *P. mandshurica* Saito, resulting in the former species becoming a synonym of the latter.

42.34. *Pichia glucozyma* (Wickerham) Kurtzman (1984a)

Synonyms:

Hansenula glucozyma Wickerham (1969b)

Ogataea glucozyma (Wickerham) Y. Yamada, Maeda & Mikata (1994c)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal or ellipsoidal, (2.5–7.1)×(2.5–7.6) μm, and single, in pairs, or in small clusters. Growth is generally mucoid and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not produced.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows no evidence of hyphae or pseudohyphae. Aerobic growth is pink from the presence of ascospores, smooth, moderately glistening, and butyrous. Colony margins are entire or lobed. A faint sweet odor may be present.

Formation of ascospores: Conjugation usually, but not always, precedes ascus formation. Generally, conjugation is between a parent cell and a bud, but may also occur between independent cells. Asci are deliquescent and produce from one to four hat-shaped ascospores. Single-spore isolates from four-spored asci form sporogenous cultures; consequently, the species is regarded to be homothallic (Kurtzman, unpublished).

Ascospores were observed on YM and 5% malt extract agars after 2–15 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+/w
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+/w
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+/w	Succinate	–
L-Arabinose	–	Citrate	+/w
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	v
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	w		

Co-Q: 7, CBS 5766 (Yamada et al. 1973a).
Mol% G+C: 45.1, CBS 5766 (T_m: Nakase and Komagata 1971b).

Origin of the strain studied: The only known strain of *P. glucozyma* was isolated from frass from an Engelmann spruce (*Picea engelmannii* Parry) growing in the Medicine Bow Mountains, Wyoming, U.S.A.

Type strain: CBS 5766 (NRRL YB-2185).

Comments: *P. glucozyma* is a particularly interesting species because of its similarity to *P. pini*. In standard tests, the species differ only in that *P. glucozyma* has the ability to assimilate nitrate. Kreger-van Rij (1964b) reported that although none of the strains of *P. pini* that she studied could assimilate nitrate, some could assimilate nitrite. Despite their great phenotypic likeness, Kurtzman (unpublished) found the type cultures of *P. glucozyma* and *P. pini* to share only 3% common nDNA base sequences.

42.35. *Pichia guilliermondii* Wickerham (1966)

Anamorph: *Candida guilliermondii* (Castellani) Langeron & Guerra

Synonyms:

Endomyces guilliermondii Castellani (1912a)
Endomycopsis guilliermondii Wickerham & Burton (1954b) nom. nud.
Yamadazyma guilliermondii (Wickerham) Billon-Grand (1989)
Monilia guilliermondii (Castellani) Castellani & Chalmers (1913)
Myceloblastanon guilliermondii (Castellani) Ota (1928)
Mycotorula guilliermondii (Castellani) Langeron & Guerra (1935) [nec *Mycotorula guilliermondii* (Castellani) Cottini & Redaelli (Cottini 1939)]
Blastodendrion guilliermondii (Castellani) Guerra (1935)
Castellania guilliermondii (Castellani) Dodge (1935)
Candida guilliermondii (Castellani) Langeron & Guerra (1938)
Endomyces negrii Castellani (1912a)
Castellania negrii (Castellani) Dodge (1935)
Monilia pseudoguilliermondii Castellani & Chalmers (1919)
Castellania pseudoguilliermondii (Castellani & Chalmers) Dodge (1935)
Monilia guilliermondii (Castellani) Castellani & Chalmers var. *pseudoguilliermondii* (Castellani & Chalmers) Castellani (1937a)
Monilia muhira Mattlet (1926)
Castellania muhira (Mattlet) Dodge (1935)
Torula fermentati Saito (1922)
Blastodendrion arzti Ota (1924a)
Monilia arzti (Ota) Nannizzi (1934)
Myceloblastanon krausi Ota (1924a)
Blastodendrion krausi (Ota) Ciferri & Redaelli (1929)
Mycotorula krausi (Ota) Redaelli & Ciferri (1947)
Microanthomyces alpinus Grüss (1926, 1927)
Candida paranensis Negroni & Fischer (1941)
Torulopsis xylinus Tatsumi & Katagiri (1950) nom. nud.
Candida melibiosi Lodder & Kreger-van Rij (1952)
Trichosporon appendiculare Batista, Silveira & Silveira (1959a)
Torulopsis carpophila M.W. Miller & Mrak (1953) nom. nud.
Candida guilliermondii (Castellani) Langeron & Guerra var. *carpophila* Phaff & M.W. Miller (1961)
Torulopsis kestonii Scarr & Rose (1966)
Candida kestonii (Scarr & Rose) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Candida guilliermondii (Castellani) Langeron & Guerra var. *soya* Onishi & Suzuki (1969b) nom. nud.
Candida parapsilosis (Ashford) Langeron & Talice var. *tokyoensis* Suzuki, Sumino, Akiyama & Fukada (1973) nom. nud.
Candida mamillae S. Goto (1979b)
Candida guilliermondii var. *japonica* Sugiyama & S. Goto (1969)
Candida parapsilosis (Ashford) Langeron & Talice var. *tuxtlensis* Herrera, Ulloa & Fuentes (1973)
Candida amidevorans Balloni, Florenzano, Mazza & Polsinelli (1987)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (1.5–4.8)×(2.0–15.0) μm, and occur singly, in pairs, or in short chains. Growth is smooth to wrinkled and tannish-white in color.

Growth on the surface of assimilation media: Generally pellicles are not formed, but rings may be present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, well-branched pseudohyphae bearing whorls of blastoconidia. True hyphae are not present. Aerobic growth is tannish-white, sometimes butyrous, smooth and glistening or dull and rugose. Margins are usually lobate.

Formation of ascospores: This species is heterothallic and only haploid forms have been isolated. Following the pairing of complementary mating types, the resulting asci produce one to four hat-shaped ascospores. The spores are liberated soon after formation.

Ascospores were observed on 5% malt extract agar at 25°C, 5–15 days after pairing of complementary mating types.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	+
Sucrose	+	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	v	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 2082 (Yamada et al. 1973a).

Mol% G+C: 44.4, 44.6 CBS 2021, CBS 2082 (T_m: Nakase and Komagata 1970b); 44.4, CBS 2030 (BD: Fuson et al. 1980).

Origin of the strains studied: Frass of American elm (*Ulmus americana*), U.S.A. (1); alcohol strain, Argentina (1); frass, Balsam fir (*Abies balsamea*), U.S.A. (1); frass, spruce (*Picea excelsa*), Germany (1); sputum (1); blood (1); atmosphere (1); unknown substrates received from Castellani (2), Langeron (1), Mackinnon (1).

Complementary mating types: NRRL Y-2075 (CBS 2030) and NRRL Y-2076 (CBS 2031).

Type strain: CBS 2030 (NRRL Y-2075), from *Ulmus americana.*

Comments: Wickerham and Burton (1954b) discovered ascosporulation in strains of *Candida guilliermondii* var. *guilliermondii* and tentatively named the sexual state *Endomycopsis guilliermondii.* Wickerham (1966) later validated the ascosporic state as *Pichia guilliermondii.* Only a small minority of natural isolates of *C. guilliermondii* are sexually reactive (Wickerham and Burton 1954b), and the type strain was not among them. Kurtzman (1992b) showed 98% nDNA complementarity between type strains of *C. guilliermondii* and *P. guilliermondii,* thus confirming the expected anamorph–teleomorph connection.

42.36. *Pichia hampshirensis* Kurtzman (1987a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are single or in pairs, spheroidal to ellipsoidal, (2.5–5.0)×(2.7–6.9) µm, and occasionally tapered. Pseudohyphae are common and moderately branched. True hyphae are not formed.

Growth on the surface of assimilation media: Pellicles are not formed, but a thin ring of surface growth may be present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only sparse formation of pseudohyphae and no true hyphae. Aerobic growth is tannish-white, slightly glistening and butyrous. A faint ester-like odor is present.

Formation of ascospores: Asci (3.0–5.0)×(5.0–6.5) µm form from vegetative cells following conjugation between a parent cell and its bud. Generally, the asci arise from budded yeast cells, but occasionally pseudohyphal cells also serve as asci. Asci are deliquescent. Ascospores are hat-shaped, (1.0–1.4)×(1.5–2.3) µm, with one to four per ascus. Single-spore isolates from four-spored asci form ascosporogenous colonies demonstrating the species to be homothallic.

Ascospores are abundant on 5% malt extract and YM agars after 3–5 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+/w
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	s	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	s
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	s	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G+C: 39.3, CBS 7208 (BD: Kurtzman 1987a).

Origin of the strain studied: NRRL YB-4128 is the only known strain of this species and was isolated in 1956, by L.J. Wickerham from the frass of a dead, cut oak (*Quercus* sp.) at Camp Sargent, New Hampshire, U.S.A.

Type strain: CBS 7208 (NRRL YB-4128).

42.37. *Pichia haplophila* Shifrine & Phaff (1956)

Synonym:
Yamadazyma haplophila (Shifrine & Phaff) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (1.5–4.2)×(2.5–8.0) µm, and occur singly, in pairs or in small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Dry, climbing pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional rudimentary pseudohyphae. True hyphae are not formed. Aerobic growth is butyrous with a dull, tannish-white almost powdery surface. The margin is finely lobed. A faint acidic odor may be present.

Formation of ascospores: Asci show conjugation between a cell and its bud or infrequently between independent cells. Two to four hat-shaped ascospores are formed by each ascus, and they are readily liberated. The predominance of conjugations between cells and their buds in ascosporogenous cultures suggests this species to be homothallic.

Ascospores were observed on 5% malt extract agar after 3–5 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	ws
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 2028 (Yamada et al. 1973a).
Mol% G+C: 39.0, 41.0, CBS 2028 (T_m: Nakase and Komagata 1970b; BD: Kurtzman, unpublished).

Origin of the strains studied: From the bark beetle *Dendroctonus jeffreyi*, U.S.A. (1); orchard soil, Japan (1).

Type strain: CBS 2028 (NRRL Y-7860), from *Dendroctonus jeffreyi*.

Comments: Results are in good agreement with previous work (Shifrine and Phaff 1956, Kreger-van Rij 1970c) except that L-sorbose assimilation was either latent or variable in the present study.

42.38. *Pichia heedii* Phaff, Starmer, Miranda & M.W. Miller (1978b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (1.8–4.1)×(3.0–6.8) µm, and occur singly, in pairs or in short chains. Growth is butyrous and cream colored.

Growth on the surface of assimilation media: Thin, climbing pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows rudimentary pseudohyphae but no true hyphae. Aerobic growth is dull, smooth, white in color, butyrous, and has a finely serrate margin. A faint acidic odor is present.

Formation of ascospores: This species is heterothallic, but only diploid strains have been isolated from nature. Vegetative cells are transformed into asci which produce two to four hat-shaped ascospores. Asci are deliquescent.

Ascospores were observed on 5% malt extract agar after 3–5 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 6930 (Billon-Grand 1985).
Mol% G+C: 32.3–32.7, CBS 6930 and 5 additional strains (BD: Phaff et al. 1978b).

Origin of the strains studied: From senita cactus (*Lophocereus schottii*), Baja California Sur, Mexico (1); saguaro cactus (*Carnegiea gigantea*), Arizona, U.S.A. (1).

Complementary mating types: NRRL Y-10968

(CBS 6931) and NRRL Y-10969 (CBS 6932), both derived from the sporogenous diploid type culture.

Type strain: CBS 6930 (NRRL Y-10967).

Comments: Phaff et al. (1978b) reported *P. heedii* to be isolated only from two species of cacti belonging to the subtribe Pachycereinae, senita (*Lophocereus schotti*) and saguaro (*Carnegiea gigantea*), and their associated *Drosophila* species, *D. pachea* and *D. nigrospiracula*, respectively. It was later found (Starmer et al. 1980, Holzschu and Phaff 1982) that the strains from senita utilize citrate whereas those isolated from saguaro do not. The two metabolic types have greater than 95% nDNA complementarity, and mating between the two types produces four-spored asci that segregate 2:2 with respect to citrate utilization, indicating control by a single genetic locus. *P. heedii* may be separated from *P. amethionina*, *P. cactophila*, *P. pseudocactophila* and *P. opuntiae* by its strong assimilation of D-xylose. Starmer et al. (1979) observed no mating among *P. heedii*, *P. amethionina* and *P. opuntiae*. *P. heedii* may be separated from *P. membranifaciens* on the basis of its failure to assimilate *N*-acetyl-D-glucosamine, its smaller cell size, comparatively slower release of ascospores and higher maximum growth temperature. The 10% difference in G+C contents between these two phenotypically similar species would preclude conspecificity.

42.39. *Pichia heimii* Pignal (1970)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (2.0–4.2)×(3.0–6.0) µm, and occur singly and in pairs. Growth is tannish-white and smooth to somewhat convoluted.

Growth on the surface of assimilation media: Moderately thick pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, moderately branched pseudohyphae with large clusters of blastoconidia. True hyphae are not formed. Infrequently, a pseudohyphal cell will be found which contains up to six endospores. Aerobic growth is tannish-white, glistening, smooth to slightly convoluted, and with a sparingly lobed margin. A faint acidic odor is present.

Formation of ascospores: Spores were not observed in the present study. Pignal (1970) reported the asci to arise from conjugation between independent cells or, less frequently, from conjugation between a cell and its bud. One to four hat-shaped ascospores were formed in each ascus, and they were liberated soon after formation.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	+	Trehalose	+
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: Not determined.

Mol% G+C: 39.7, CBS 6139 (T_m: J.B. Fiol, cited by Billon-Grand 1981).

Origin of the strain studied: Decaying, insect-invaded wood, Equatorial Africa.

Type strain: CBS 6139 (NRRL Y-7502).

Comments: The results of the present study are in agreement with the original description except that Pignal (1970) reported a weak fermentation of raffinose and weak growth on L-sorbose, which were not detected.

42.40. *Pichia henricii* (Wickerham) Kurtzman (1984a)

Synonyms:
Hansenula henricii Wickerham (1969b)
Ogataea henricii (Wickerham) Y. Yamada, Maeda & Mikata (1994c)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal or ellipsoidal, (3.1–5.4)×(3.4–7.5) µm, and occur singly, in pairs, and in small clusters. Growth is butyrous and tannish-white in color. Growth from some single-ascospore isolates can be mucoid.

Growth on the surface of assimilation media: Neither pellicles nor rings are produced.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Occasional short chains of cells are observed. Aerobic growth is tannish-white, smooth, faintly glistening, and butyrous. Colony margins are entire.

Formation of ascospores: Asci may be unconjugated or arise from conjugation between independent cells or a parent cell and its bud. Ascospores are hat-shaped and

the asci are deliquescent. Usually only two spores are formed per ascus on YM- or 5% malt extract agar, but on morphology agar, three- or four-spored asci are common. The species apparently is homothallic because single-spore isolates from four-spored asci give ascosporogenous colonies (Kurtzman, unpublished).

Ascospores were observed on YM, 5% malt extract, and yeast morphology agars after 10–20 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	w
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	w/–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	+/w
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	w/–		

Co-Q: 7, CBS 5765 and 2 additional strains (Yamada et al. 1973a).

Mol% G+C: 49.8–50.2, CBS 5765, AJ 5164, AJ 5165 (T_m: Nakase and Komagata 1971b); 49.6, CBS 5765 (BD: Fuson et al. 1979).

Origin of the strains studied: Bird (?) feces collected in the Medicine Bow Mountains, Wyoming, U.S.A. (1); frass from a lodgepole pine (*Pinus contorta* Douglas) collected near Markleeville, California, U.S.A. (1); soil, Medicine Bow Mountains, Wyoming (1).

Type strain: CBS 5765 (NRRL YB-2194), from bird (?) feces, Medicine Bow Mountains, Wyoming.

42.41. *Pichia holstii* (Wickerham) Kurtzman (1984a)

Anamorph: *Candida silvicola* Shifrine & Phaff

Synonyms:

Hansenula holstii Wickerham (1960)

Nakazawaea holstii (Wickerham) Y. Yamada, Maeda & Mikata (1994c)

Candida silvicola Shifrine & Phaff (1956)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ellipsoidal to elongate, (1.7–4.0)×(2.1–6.9) μm, and single, in pairs, or in small clusters. Growth is mucoid to butyrous and white in color.

Growth on the surface of assimilation media: Infrequently, thin pellicles or rings may be formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and well-branched pseudohyphae as well as true hyphae. Aerobic growth is white to tannish-white, dull to glistening, and mucoid to butyrous. Colony margins are entire or lobed and generally bordered by pseudohyphae and true hyphae. Freshly isolated strains sometimes have cells that produce tapered outgrowths at one or both ends. The outgrowths may attain a length of several times that of the main portion of the cell. A faint ester-like odor is produced.

Formation of ascospores: *P. holstii* is heterothallic, and only asporogenous haploid strains have been isolated from nature. Asci are formed following conjugation of complementary mating types, and they contain two to four hat-shaped ascospores. The asci are deliquescent. Many natural isolates lack mating competence or show a very weak reaction (Wickerham 1960, Kurtzman et al. 1973a). Following mixture of complementary mating types, 2 or 3 weeks may pass before conjugants are observed. Few isolates from frass of *Pinus* show mating competence, but a much higher percentage of isolates from gums of *Prunus* spp. are sexually reactive. Herman (1971a) reported increased mating frequency and increased sporulation in shaken cultures employing a minimal medium developed to enhance extracellular polymer production (Slodki et al. 1972).

Ascospores were observed at 25°C on 5% malt extract agar and in shaken cultures of polymer production medium, sometimes after only 5–10 days.

Fermentation:

Glucose	+	Lactose	–
Galactose	w/–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	+/w		

Co-Q: 8, CBS 4140, CBS 4069 (Yamada et al. 1973a).
Mol% G+C: 36.8, 37.1, CBS 4140 (T_m: Nakase and Komagata 1971b; BD: Kurtzman, unpublished).

Origin of the strains studied: Several genera of coniferous and broadleaf trees from throughout the U.S. and Canada as well as from lichens, soil, lakes, and streams (154); cadaver (1).

Complementary mating types: NRRL Y-2154 (CBS 4141) and NRRL Y-2155 (CBS 4140).

Type strain: CBS 4140 (NRRL Y-2155), isolated by Wickerham (1960) from frass of an apparent yellow spruce (*Picea rubens*?) from the Gaspé Peninsula, Canada.

Comments: As with *P. capsulata*, *P. holstii* produces large quantities of extracellular polysaccharides. Slodki and coworkers (Slodki et al. 1972, Seymour et al. 1976) have shown the mannans and phosphomannans of these two species to be different.

42.42. *Pichia inositovora* Golubev & Blagodatskaya (Golubev et al. 1981)

Synonym:
Yamadazyma inositovora (Golubev & Blagodatskaya) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are short ovoidal to elongate, (2.1–4.5)×(2.5–7.6) μm, and single or infrequently in pairs. Colonies are butyrous, glistening and tannish-white.

Growth on the surface of assimilation media: Thin climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is tannish-white, glistening, butyrous, and low convex in profile with a depressed center. Colony margins are entire to infrequently lobed. A faint musky-acidic odor is produced.

Formation of ascospores: Asci are unconjugated and form one to four hat-shaped ascospores that are released at maturity (Fig. 139). It is not known whether the species is homothallic or heterothallic. Golubev et al. (1981) observed from transmission electron microscopy that bulb-like structures sometimes form on the brims of ascospores.

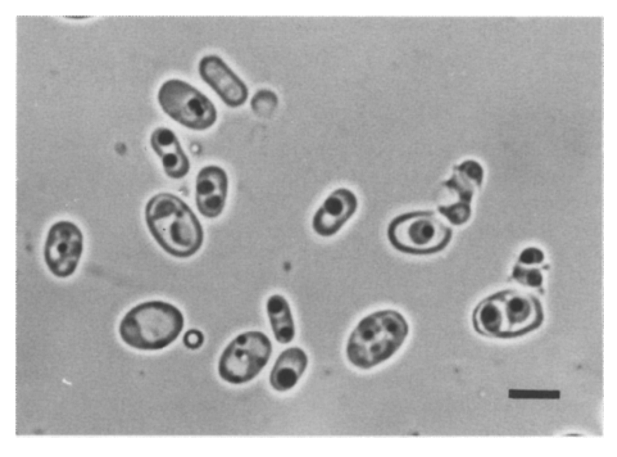


Fig. 139. *P. inositovora*, CBS 8006. Ascosporogenous culture after 3 weeks, 15°C, on YM agar. Bar = 5 μm.

Ascospores were observed on YM and 5% malt extract agars after 2–3 weeks at 15°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	l	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	l	Inositol	+
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 9, CBS 8006 (Billon-Grand 1985).
Mol% G+C: 49.9, CBS 8006 (BD: Kurtzman, unpublished).

Origin of the strain studied: The only known strain of this species was isolated from highmoor peat in the Moscow region, Russia.

Type strain: CBS 8006 (IBPhM Y-888, NRRL Y-12698).

Comments: Results from the present study are in agreement with the original description. The only exception was lactate assimilation, which was absent in the present examination but reported to be positive but slow by Golubev et al. (1981). *P. inositovora* is one of only a few

ascomycetous yeasts that assimilate *i*-inositol as a carbon source.

42.43. *Pichia jadinii* (A. & R. Sartory, Weill & J. Meyer) Kurtzman (1984a)

Anamorph: *Candida utilis* (Henneberg) Lodder & Kreger-van Rij

Synonyms:

Saccharomyces jadinii A. & R. Sartory, Weill & J. Meyer (1932)
Hansenula jadinii (A. & R. Sartory, Weill & J. Meyer) Wickerham (1951)
Torula utilis Henneberg (1926)
Torulopsis utilis (Henneberg) Lodder (1934)
Cryptococcus utilis (Henneberg) Anderson & Skinner (1947)
Candida utilis (Henneberg) Lodder & Kreger-van Rij (1952)
?*Torula mineralis* Hayduck & Haehn ex F.C. Harrison (1928)
Candida guilliermondii (Castellani) Langeron & Guerra var. *nitratophila* Diddens & Lodder (1942)
Torulopsis utilis (Henneberg) Lodder var. *major* Thaysen & Morris (1943)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ellipsoidal to elongate, (2.5–8.0)×(4.1–11.2) μm, and occur singly or in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin pellicles may form infrequently on some media.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional pseudohyphae and these may be highly branched. True hyphae are not produced. Aerobic growth is white to tannish-white, smooth, glistening, and butyrous. Colony margins are entire or infrequently lobed. A faint ester-like odor may be produced.

Formation of ascospores: *P. jadinii* forms one to four hat-shaped ascospores in unconjugated deliquescent asci (Fig. 140). Kurtzman et al. (1979) showed that single-spore isolates from the type strain either sporulated no better than the parent or not at all. Because the parent culture sometimes failed to sporulate and because mixtures of asporogenous single-spore isolates did not sporulate, it was concluded that *P. jadinii* is homothallic.

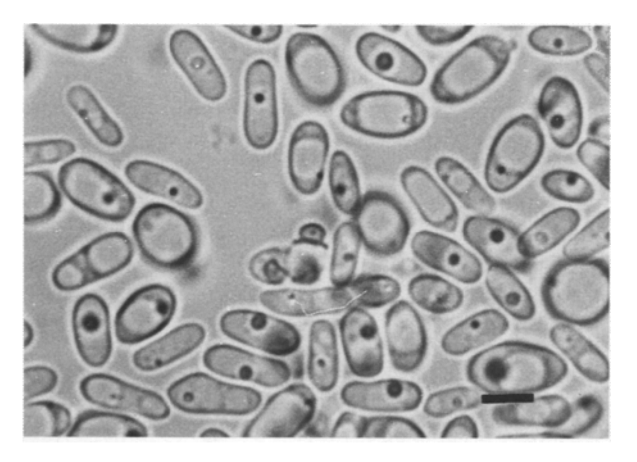


Fig. 140. *P. jadinii*, CBS 1600. Ascosporogenous culture after 2 weeks, 25°C, on a carrot wedge. Bar = 5 μm.

Ascospores were observed on 5% malt extract agar and on sterilized carrot slices after 10–30 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+/w	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	w/–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 1600 (Yamada et al. 1973a).

Mol% G + C: 43.2, CBS 1600 (T_m: Nakase and Komagata 1971b); 45.1–45.8, CBS 1600, 5609, 621 (BD: Kurtzman et al. 1979).

Origin of the strains studied: Pus from a human abscess (1), the udder of a cow with mastitis (1), industrial fermentations (4).

Type strain: CBS 1600 (NRRL Y-1542), isolated by Sartory et al. (1932) from the pus of a human abscess.

Comments: *Candida utilis* had been proposed as the asexual state of *P. jadinii* because of phenotypic similarities (Wickerham 1970a). DNA reassociation studies demonstrated that *C. utilis* and *P. jadinii* share 85% common base sequences (Kurtzman et al. 1979). These data, confirmed by Manachini (1979), show *C. utilis* to be the anamorphic form of *P. jadinii*. By contrast, *P. petersonii* showed only 9% DNA relatedness with *C. utilis* and *P. jadinii* and, therefore, must be considered a separate species.

The possibilities of growing *C. utilis* (Torula yeast) as a foodstuff on a commercial scale were first recognized by German workers in Berlin at the Institut für Gärungsgewerbe during World War I (Lindner 1922, as cited by Pyke 1958). Because *C. utilis* is capable of utilizing pentoses, pulping-waste liquors from the paper industry have been used for about four decades as an economical substrate for culturing this yeast. The general acceptance of *C. utilis* by the food and feed industries

as a safe and nutritious form of single-cell protein has made it an important species for cultivation on other types of biological wastes. Because of its importance to the food industry, the habitats of *P. jadinii* and *C. utilis* deserve further comment. One of the two known strains of *P. jadinii* was isolated from abscesses of a young woman and the second strain was obtained from a localized infection in a cow. *C. utilis* has been isolated from flowers and as a contaminant in industrial fermentations, but also from human and animal sources. In view of this, strains of *C. utilis* that have a long history of safety need to be used when Torula yeast is produced. Three strains of *C. utilis* used commercially since at least the mid-1940's are NRRL Y-900 (CBS 5609), NRRL Y-1082 (CBS 1517), and NRRL Y-1084 (CBS 841).

42.44. *Pichia japonica* Kurtzman (1987a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.2–5.0)×(2.8–8.5) µm, and single or in pairs. Branched pseudohyphae are present, but true hyphae are not formed.

Growth on the surface of assimilation media: Pellicles are not formed, but a thin ring of surface growth may be present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows moderately branched pseudohyphae with straight or curved cells. True hyphae are not present. Aerobic growth is grayish-white, glistening, butyrous to slightly mucoid and fringed with pseudohyphae. A faint ester-like odor is present.

Formation of ascospores: Asci (3.0–4.0)×(4.5–7.0) µm form from vegetative cells following conjugation between a parent cell and its bud. Asci arise from budded yeast cells or occasionally from pseudohyphal cells. The asci are deliquescent. Ascospores are hat-shaped and one to four occur in each ascus (Fig. 141). Single-spore isolates from four-spored asci give ascosporogenous colonies indicating the species to be homothallic.

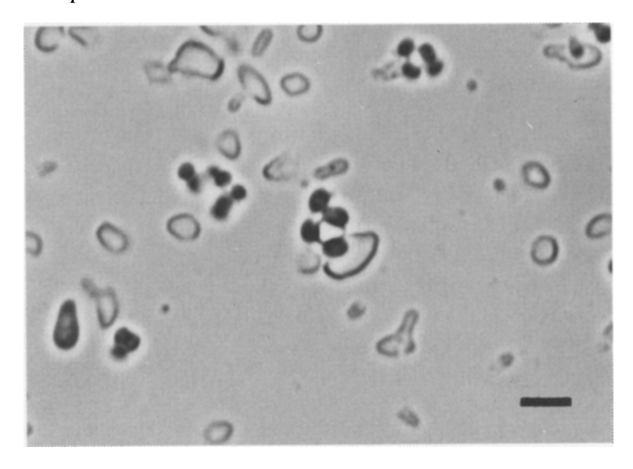


Fig. 141. *P. japonica*, CBS 7209. Ascosporogenous culture after 3 days, 25°C, on 5% malt extract agar. Bar = 5 µm.

Ascosporulation is abundant on 5% malt extract agar and only slightly less so on YM agar, after 7–10 days at 25°C.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+/w
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+/w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–	Growth at 40°C	+

Co-Q: Not determined.

Mol% G + C: 46.4–46.7, CBS 7209, 7210, 7211, 7212 (BD: Kurtzman 1987a).

Origin of the strains studied: All four known strains of this species [NRRL YB-2750 (CBS 7209), YB-2751 (CBS 7210), YB-2752 (CBS 7211) and YB-2753 (CBS 7212)] were isolated in 1951 by L.J. Wickerham from a frass sample from fir (*Abies firma*) sent by the Ministry of Agriculture and Forestry, Tokyo, Japan.

Type strain: CBS 7209 (NRRL YB-2750)

Comments: Comparisons of nDNA complementarity between *P. japonica*, *P. hampshirensis* and other phenotypically similar species of *Pichia* and *Candida* showed *P. japonica* to be a genetically distinct species (Kurtzman 1987a).

42.45. *Pichia kluyveri* Bedford ex Kudryavtsev (1960)

This species has three varieties:

Pichia kluyveri Bedford ex Kudryavtsev (1960) var. *kluyveri* (1987)

Synonyms:

Pichia kluyveri Bedford (1942) nom. nud.

Hansenula kluyveri (Bedford) Kudryavtsev (1960)

Pichia belgica (Lindner) Dekker var. *microspora* Negroni & Fischer (1941) nom. nud.

***Pichia kluyveri* var. *cephalocereana* Phaff, Starmer & Tredick-Kline (1987b)**

***Pichia kluyveri* var. *eremophila* Phaff, Starmer & Tredick-Kline (1987b)**

Anamorph: *Candida eremophila* Phaff, Starmer & Tredick-Kline
Synonym:
Candida eremophila Phaff, Starmer & Tredick-Kline (1987b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2.0–5.5)×(4.0–11.0) μm, and occur singly, in pairs, or in short chains. Growth is yellowish-tan, dull and with fine wrinkles.

Growth on the surface of assimilation media: Dry, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows moderately well-branched pseudohyphae, but they produce few blastoconidia. True hyphae are not formed. Aerobic growth is tannish-white, dull, sometimes almost powdery, and with fine striations radiating from center to colony edge. Margins are entire to finely serrate.

Formation of ascospores: Two to four hat-shaped spores are produced in each ascus, and they are released very soon after formation (Fig. 142). Phaff et al. (1987b) reported that most strains are heterothallic but some, including the two-spored strains, appear homothallic.

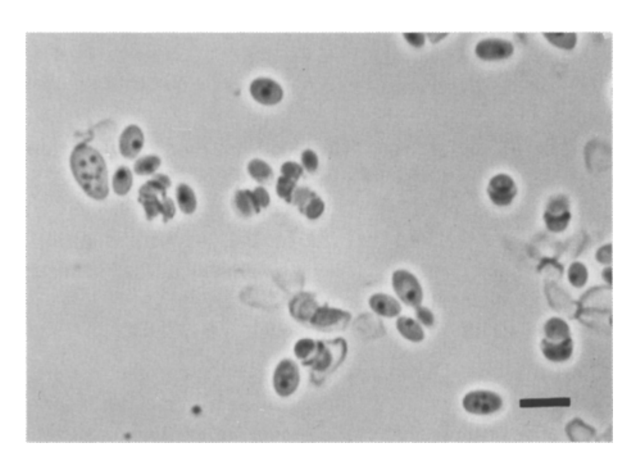


Fig. 142. *P. kluyveri* var. *kluyveri*, CBS 1525. Ascosporogenous culture after 3 days, 25°C, on YM agar. Bar = 5 μm.

Ascospores were observed on McClary's acetate, YM and 1% malt extract agars, usually after 5–15 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	s
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+/w
D-Xylose	v	Succinate	+/w
L-Arabinose	–	Citrate	w/–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	+		

Co-Q: 7, AJ 4139, AJ 4145 (Yamada et al. 1973a).
Mol% G+C: 28.3–28.5, AJ 4145, AJ 4426, var. *kluyveri* (T_m: Nakase and Komagata 1970b); 29.4–31.4, CBS 188 and 7 additional strains, var. *kluyveri* (BD: Phaff et al. 1987b); 29.9–30.5, CBS 7273 and 3 additional strains, var. *cephalocereana* (BD: Phaff et al. 1987b); 29.8–30.8, CBS 7272 and 3 additional strains, var. *eremophila* (BD: Phaff et al. 1987b); 29.4–30.4, CBS 7271 and 3 additional strains, *Candida eremophila* (BD: Phaff et al. 1987b).

Supplementary description of *P. kluyveri* varieties: Variety *kluyveri*: glucose fermentation is strong; citrate assimilation is weak or absent: killer activity against *Candida glabrata*; rare in cacti, common in rotting fruit of other plants (Phaff et al. 1987b, Starmer et al. 1992).

Variety *cephalocereana*: glucose fermentation is slow and usually late; citrate is assimilated; no killer activity against *C. glabrata*; from columnar cacti on the Caribbean island of Montserrat (Phaff et al. 1987b).

Variety *eremophila*: glucose fermentation is weak or absent; citrate is assimilated; killer activity against *C. glabrata*; nearly exclusive to *Opuntia* cactus rots in southern Arizona and Texas (Phaff et al. 1987b).

Origin of the strains belonging to the variety *kluyveri*: Olives, Bedford (1); *P. belgica* var. *microspora* (CBS 1358), P. Negroni; tropical products (1); fermenting cacao (2); *Drosophila* sp. from tomato (2); prickly pear cactus (*Opuntia stricta*) (2).

Complementary mating types: CBS 7907 [SU (Syracuse University) 84-670.2B, NRRL Y-17734 (h^+)] and CBS 7908 [SU 84-670.2C, NRRL Y-17752 (h^-)].

Type strain: CBS 188 (NRRL Y-11519), from olives.

Origin of the strains belonging to the variety *cephalocereana*: Rots of columnar cactus (*Cephalocereus royenii*), island of Montserrat (2).

Complementary mating types: CBS 7909 [SU 82-555B.17, NRRL Y-17718 (h^+)] and CBS 7910 [SU 82-556D.30, NRRL Y-17738 (h^-)].

Type strain: CBS 7273 (SU 82-555B, NRRL Y-17225).

Origin of the strains belonging to the variety *eremophila*: Rots of prickly pear cacti: *Opuntia phaeacantha*, southern Arizona, USA (1); *Opuntia* sp., Baja California, Mexico (1). Rotting organ pipe cactus (*Stenocereus thurberi*), Baja California, Mexico (1); type strain of *Candida eremophila* (CBS 7271, UCD-FST 76-202B, NRRL Y-17226).

Complementary mating types: CBS 7906 [SU 83-466.1AC, NRRL Y-17753 (h^+)] and CBS 7905 [SU 86-541.5A, NRRL Y-17751 (h^-)].

Type strain: CBS 7272 (SU 83-466.1, NRRL Y-17224), from *Opuntia phaeacantha*.

Comments: Following an extensive survey of the yeast flora of rotting cacti, Phaff et al. (1987b) demonstrated three genetically divergent populations among isolates of *P. kluyveri*. Nuclear DNA relatedness within populations was high, but intergroup nDNA relatedness ranged from 66–72%. A majority of the strains that produced four-spored asci from each of the populations was demonstrated to be heterothallic through single-ascospore isolations. Fertility of progeny from intergroup crosses appeared somewhat diminished. Because of reduced nDNA relatedness and reduced fertility between the groups, Phaff et al. (1987b) proposed that the groups represented taxonomic varieties. A fourth group of strains that was isolated from *Stenocereus* rots had high nDNA relatedness with the variety *eremophila* but failed to show any mating response. This group was described as *Candida eremophila*. Separation of *P. kluyveri* var. *eremophila* from *P. galeiformis* and *P. membranifaciens* is discussed in the Comments section of the latter species.

42.46. *Pichia kodamae* van der Walt & Yarrow (van der Walt et al. 1982)

Synonym:
Ogataea kodamae (van der Walt & Yarrow) Mikata & Y. Yamada (1995)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal or infrequently elongate, (1.5–5.6)×(1.5–6.3) µm, and single, in pairs or in small clusters. Growth is mucoid, glistening and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass is devoid of either pseudohyphae or hyphae. Aerobic growth is tannish-white, mucoid, glistening, convex in profile and with an entire margin. A musky, acidic odor is present.

Formation of ascospores: Asci may be unconjugated or show parent cell–bud conjugation. The asci produce one to four hat-shaped ascospores and become deliquescent at maturity. Van der Walt et al. (1982) reported the species to be homothallic.

Ascosporulation was observed on YM and 5% malt extract agars after 5–7 days at 25°C.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	ws
L-Sorbose	v	Ethanol	ws
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	w/–
Soluble starch	–	DL-Lactate	–
D-Xylose	s	Succinate	s
L-Arabinose	v	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	w
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 7081 (Billon-Grand 1985).
Mol% G + C: Not determined.

Origin of the strain studied: CBS 7081 (NRRL Y-17234), from insect infestations of spurge (*Euphorbia ingens*), Groblersdal district, South Africa.

Type strain: CBS 7081.

Comments: Methanol assimilation by *P. kodamae* appears somewhat variable. In the original description, van der Walt et al. (1982) reported methanol not to be assimilated, whereas Barnett et al. (1990) reported a delayed assimilation of this compound. In the present study, growth on methanol was weak and slow.

Van der Walt et al. (1982) noted *P. kodamae* to be phenotypically similar to *P. methanolica* and *P. pini*. Their attempts to induce mating between auxotrophic mutants of *P. kodamae* and the other two species failed, which suggested *P. kodamae* to be a separate species.

42.47. *Pichia lynferdii* (van der Walt & E. Johannsen) Kurtzman (1984a)

Synonyms:
Hansenula lynferdii van der Walt & E. Johannsen (1975b)
Candida lynferdii van der Walt & E. Johannsen (1975b)

Growth on 5% malt extract agar: After 3 days

at 25°C, the cells are spheroidal to ellipsoidal, (2.1–5.8)×(2.7–7.5) µm, and single or in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin creeping pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional moderately well-developed pseudohyphae. True hyphae are not formed. Aerobic growth is tannish-white, slightly glistening, and butyrous. Colony margins are entire to finely lobed. A faint acidic odor is produced.

Formation of ascospores: Asci are unconjugated and arise directly from vegetative cells. There are one to four hat-shaped ascospores per ascus, and asci are deliquescent. Van der Walt and Johannsen (1975b) reported that heat-treatment of sporogenous cultures gave only sporogenous colonies suggesting the species to be homothallic.

Ascospores were observed on YM, 5% malt extract and V8 agars after 5–7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: Not determined.
Mol% G+C: 39.3, CBS 6695 (HPLC: Miller and Baharaeen 1979).

Origin of the strain studied: The only known strain of this species was recovered from uncultivated grassland soil collected near Pretoria, South Africa.

Type strain: CBS 6695 (NRRL Y-7723), isolated from soil in South Africa and received from van der Walt.

Comments: *Pichia lynferdii* shares many characteristics with *P. jadinii* and *P. petersonii* including a tolerance to embalming fluid (van der Walt and Johannsen 1975b). The three species can be separated by their assimilation reactions on L-rhamnose, galactose, and erythritol. Van der Walt and Johannsen (1975b) reported *P. lynferdii* to give slow, weak growth on L-arabinose, but in the present study, no growth was detected on this compound.

42.48. *Pichia media* Boidin, Pignal, Lehodey, Vey & Abadie (1964)

Synonym:
Yamadazyma media (Boidin, Pignal, Lehodey, Vey & Abadie) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (1.9–3.5)×(3.0–5.5) µm, and occur singly or in pairs. Growth is yellowish-white, smooth, dull-glistening, and butyrous.

Growth on the surface of assimilation media: Pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is light tannish-white, smooth, or with minute striations, dull-glistening, butyrous, and with a smooth to finely lobed margin.

Formation of ascospores: Conjugation between parent cell and bud or occasionally between independent cells precedes ascus formation. Asci contain one to four hat-shaped ascospores which are liberated soon after formation. Based on manner of conjugation, it is expected that this species is homothallic.

Ascospores were observed on 5% malt extract agar after 5–10 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	w/–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	w/–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 5521 (Billon-Grand 1985).

Mol% G+C: 36.1, CBS 5521 (T_m: J.B. Fiol, cited by Billon-Grand 1981).

Origin of the strains studied: Xylophagous insect larvae, France (2).

Type strain: CBS 5521 (NRRL Y-7122).

Comments: Kreger-van Rij (1970c) noted some inconsistencies in assimilation between her tests and those of the original description. In turn, some differences with her results were found in the present study. This may reflect differences in the purity of the carbon compounds used or more likely variability within *P. media* itself. In contrast to the 1970 study, assimilation of soluble starch was negative or weak, L-arabinose was positive rather than variable, D-ribose was positive but sometimes slow, D-arabinose was positive rather than variable, and galactitol was variable rather than negative.

42.49. *Pichia membranifaciens*[1] (E.C. Hansen) E.C. Hansen (1904)

Anamorph: *Candida valida* (Leberle) van Uden & H.R. Buckley, on the basis of phenotypic similarity.

Synonyms:

Saccharomyces membranifaciens (as *S. membranaefaciens*) E.C. Hansen (1888a)
?*Saccharomyces pyriformis* Ward (1892)
Saccharomyces anomalus E.C. Hansen var. *belgicus* Lindner (1895)
?*Saccharomyces hyalosporus* Lindner (1901)
?*Pichia hyalospora* (Lindner) E.C. Hansen (1904)
Willia belgica Lindner (1905a)
Hansenula belgica (Lindner) H. & P. Sydow (1919)
Endomyces belgica (Lindner) Zender (1925b)
Pichia belgica (Lindner) Dekker (Stelling-Dekker 1931)
Pichia membranaefaciens E.C. Hansen var. *belgica* (Lindner) K. Kodama, Kyono & S. Kodama (1955)
Mycoderma valida Leberle (1909)
Mycokluyveria valida (Leberle) Ciferri & Redaelli (1947)
Mycoderma decolorans Will (1910)
Mycokluyveria decolorans (Will) Ciferri & Redaelli (1947)
Saccharomyces mycoderma punctisporus Mélard (1910)
Pichia punctispora (Mélard) Dekker (Stelling-Dekker 1931)
Pichia alcoholophila Klöcker (1912a)
Pichia calliphorae Klöcker (1912a)
Pichia membranaefaciens E.C. Hansen var. *calliphorae* (Klöcker) Dekker (Stelling-Dekker 1931)
Mycoderma vanlaeriana Lindner & Genoud (1913)
Candida krusei (Castellani) Berkhout var. *vanlaeriana* (Lindner & Genoud) Diddens & Lodder (1942)
Pichia mandshurica Saito (1914)
Pichia membranaefaciens E.C. Hansen var. *mandshurica* (Saito) K. Kodama, Kyono & S. Kodama (1955)
Zygosaccharomyces chevalieri Guilliermond (1914)
Zygopichia chevalieri (Guilliermond) Klöcker (1924)
Zygosaccharomyces bisporus Anderson (1917)
Mycoderma tannica Asai (1918)
Mycokluyveria tannica (Asai) Ciferri & Redaelli (1947)
Endomyces chodati Zender (1925a)
Willia chodati (Zender) Guilliermond (1928)
Pichia chodati (Zender) Dekker (Stelling-Dekker 1931)
Endomyces trumpyi Zender & Bevan (Zender 1925a)
Pichia chodati (Zender) Dekker var. *trumpyi* (Zender & Bevan) Dekker (Stelling-Dekker 1931)
Willia trumpyi (Zender) Guilliermond (Stelling-Dekker 1931)
Mycoderma lafarii Janke (1930)
Mycokluyveria lafarii (Janke) Ciferri & Redaelli (1947)
Pichia neerlandica Lodder (1932)
Pichia alcoholophila Klöcker var. *naganishii* Lodder (1932)
Zygopichia guilliermondii Naganishi (1933)
Pichia derossii Castelli (1935) nom. nud.
Zygopichia chiantigiana Castelli (1938)
Mycoderma vini-lafarii Zimmermann (1938)
Pichia membranaefaciens E.C. Hansen var. *acidificans* Scrivani (1939)
Pichia chodati (Zender) Dekker var. *fermentans* Mrak, Phaff & Vaughn (1942b)
Zygowillia chodati (Mrak, Phaff & Vaughn) Kudryavtsev (1960)
?*Pichia fermentans* var. *rugosa* Bedford (1942) nom. nud.
Torulopsis californicus Mrak & McClung (1940) nom. nud.
Cryptococcus californicus Mrak & McClung ex Anderson & Skinner (1947)
?*Pichia pulque* Guilliermond (Krasil'nikov 1954a)
Zygopichia chevalieri (Guilliermond) Klöcker var. *andersonii* Nickerson (1944b)
Pichia silvestris Phaff & Knapp (1956)
Pichia membranaefaciens E.C. Hansen var. *sicereum* Santa María (1956c)
Pichia indica Bahadur (1957) nom. nud.
Pseudohansenula indica (Bahadur) Novák & Zsolt (1961)
Pichia saccharophila Sasaki & Yoshida (1959)
Pichia farinosa (Lindner) E.C. Hansen var. *lodderi* Negroni & Fischer, nom. nud.? (cited in Kreger-van Rij 1970c)
Candida valida (Leberle) van Uden & H.R. Buckley (1970)
Pichia scaptomyzae C. Ramírez & González (1984e)
Pichia miyazi Mikata & Banno (1985) nom. nud.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (1.8–4.5)×(2.5–17.0) μm, and occur singly, in pairs, in chains, or in clusters. Growth is yellowish-tan, dull and smooth or wrinkled.

Growth on the surface of assimilation media: Dry, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass generally shows moderately branched pseudohyphae, although some strains may form none. True hyphae are not produced. Aerobic growth is tannish-white, dull, smooth to wrinkled and with an irregular lobed margin. A faint acidic odor may be present.

Formation of ascospores: Asci may be unconjugated or show conjugation between independent cells or between a parent cell and a bud. One to four ascospores are usually formed per ascus, but Santa María (1960, 1966) has observed as many as seven. Ascospores may be spheroidal or hemispheroidal and with or without a ledge (Figs. 143,

[1] The original spelling of the species epithet *membranaefaciens* has been treated as an orthographic error.

144). Ascospores with ledges may appear nearly hat-shaped or nearly saturn-shaped. When viewed in the scanning electron microscope, it is not uncommon to find a single strain producing the whole range of spore shapes. Spores are generally liberated from the ascus. Single-spore isolates from four-spored asci show some cultures to be homothallic, but Slooff (1964) and Kreger-van Rij (1970c) clearly demonstrated other strains to be heterothallic. Of further interest was Kreger-van Rij's (1970c) observation of enhanced sporulation in certain self-sporulating strains following their conjugation with one or the other of the mating types.

Ascospores were observed on YM, 5% malt extract, and V8 agars after 2–15 days at 25°C.

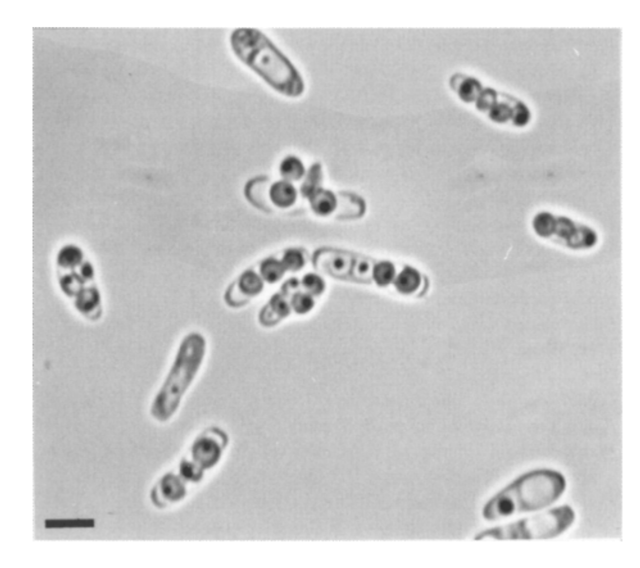


Fig. 143. *P. membranifaciens*, CBS 1329. Ascosporogenous culture after 12 days, 25°C, on YM agar. Bar = 5 µm.

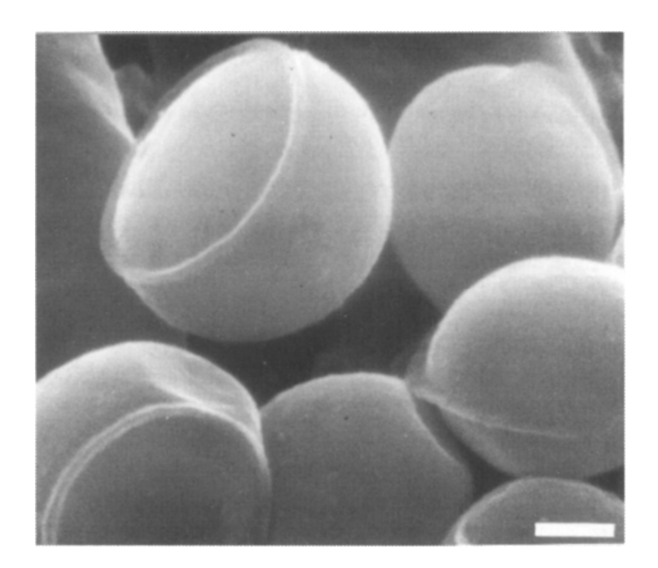


Fig. 144. *P. membranifaciens*, CBS 1329. Scanning electron micrograph showing placement of ledges on ascospores to be saturn-like, hat-like, or incomplete. Bar = 0.5 µm.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	v	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	+		

Co-Q: 7, AJ 4110, AJ 4113, AJ 4140 (Yamada et al. 1973a).
Mol% G + C: 41.5–42.4, IFO 0460 and 5 additional strains (T_m: Nakase and Komagata 1970b); 44.3, CBS 107 (BD: Phaff et al. 1976).

Origin of the strains studied: Grape must (1); wine (1); vineyard soil (2); beer (1); raw material for sorghum brandy (1); lambic gueuse (1); feces (1); maize-cattle waste fermentation (3); unknown substrates (9).

Complementary mating types: CBS 5567 (NRRL Y-6775) and CBS 5568 (NRRL Y-6776), from Slooff (1964).

Type strain: CBS 107 (NRRL Y-2026).

Comments: Strains of *P. membranifaciens* show considerable variation in the amount of pseudomycelium produced, in the assimilation of several carbon compounds, in growth at 37°C and in their requirement for vitamins. However, there seems little correlation among the varying characteristics that would allow sub-grouping of the strains, and Kreger-van Rij (1970c) found mating between strains showing differences in one or more of these properties. Consequently, all of this phenotypic variation is considered typical for the species.

Reliance on phenotypic characters for the separation of *P. membranifaciens* from *P. galeiformis* and *P. kluyveri* var. *eremophila* will result in misidentification of some strains (Table 32). *P. kluyveri* var. *eremophila* is not known outside cactus habitats, but *P. membranifaciens* is cosmopolitan and the habitat range of *P. galeiformis* is insufficiently known. Strains that assimilate L-sorbose can be identified as *P. membranifaciens*, but the species

is variable for this character. Heavily sporulated cultures of *P. membranifaciens* are unique among the three taxa because they produce a noticeable reddish-brown color, but lightly sporulating strains cannot be discerned by this character. When strain identification is critical, molecular comparisons will be required.

42.50. *Pichia methanolica* Makiguchi (Kato et al. 1974)

Synonyms:
Pichia aganobii Urakami & Michimi (1977) nom. nud.
Pichia cellobiosa J.-D. Lee & Komagata (1980b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (1.9–6.0)×(2.0–8.8) µm, and occur singly, in pairs or in clusters. Growth is cream-colored, smooth, glistening and butyrous.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is pale tannish-white, smooth, glistening and butyrous with an entire to lobed margin. Some strains produce a faint odor of esters.

Formation of ascospores: Asci may show parent–bud conjugation or conjugation between independent cells. One to four hat-shaped spores are produced in each ascus, and the spores are freed soon after formation. Because conjugations between a cell and its bud are common in ascosporogenous cultures, this species appears to be homothallic.

Ascospores were observed on 5% malt extract agar and Difco yeast morphology agar after 4–7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	+
L-Sorbose	v	Ethanol	+
Sucrose	w/–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+/w	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7 and 8 (nearly equal amounts), CBS 6515, type strain of *P. methanolica* (Lee and Komagata 1983); 7/8, CBS 8002, type strain of *P. cellobiosa* (Lee and Komagata 1980b).

Mol% G+C: 36.9, CBS 8002 (T_m: Lee and Komagata 1980b); 36.4, CBS 6515 (T_m: Billon-Grand 1981).

Origin of the strains studied: Soil, Japan (7): *P. methanolica* CBS 6515 (type strain) and one additional strain; *P. cellobiosa* CBS 8002 (type strain) and two additional strains; *P. aganobii* CBS 8053 (representative strain) and one additional strain.

Type strain: CBS 6515 (NRRL Y-7685).

Comments: Some differences were noted in the results obtained from fermentation and assimilation tests in the present study and those reported by Kato et al. (1974). In contrast to the original description, maltose was not fermented; however, L-sorbose and D-arabinose were assimilated, but citric and lactic acids were not utilized. Furthermore, growth was absent in vitamin-free medium. Kato et al. (1974) reported this species to be noteworthy because of its strong assimilation of methanol.

Lee and Komagata (1983) demonstrated that the electrophoretic profiles of 13 cellular enzymes were identical for *P. methanolica* and *P. cellobiosa* and proposed the two taxa to be conspecific. Kurtzman (1992b) showed 100% nDNA complementarity between type strains of *P. methanolica* and *P. cellobiosa* providing additional evidence that the two represent a single species. In other nDNA comparisons, *P. aganobii* showed 100% complementarity with *P. methanolica* (Kurtzman and Robnett, unpublished data).

42.51. *Pichia methylivora*[2] Kumamoto & Seriu (Kumamoto et al. 1986)

Anamorph: *Candida cariosilignicola* J.-D. Lee & Komagata
Synonym:
Candida cariosilignicola J.-D. Lee & Komagata (1980b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.3–3.5)×(1.9–4.1) µm, and single or in pairs. Growth is mucoid, glistening and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae, but occasional "tree-like" outgrowths of undifferentiated cells develop. Aerobic growth is tannish-white, glistening, mucoid and convex in

[2] The original spelling of the species epithet *methylovora* has been treated as an orthographic error.

profile with a smooth margin. A faint musky, acidic odor is present.

Formation of ascospores: Ascospores were not observed in the present study. Kumamoto et al. (1986) presented photographs of hat-shaped ascospores and reported that two were produced per ascus on 5% malt extract agar after 7 days. Asci are deliquescent. It was not reported whether the species is homothallic or heterothallic.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	+
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7 (Kumamoto et al. 1986).
Mol% G+C: 35.8, CBS 7300 (T_m: Kumamoto et al. 1986).

Origin of the strains studied: CBS 7300 (NRRL Y-17250), isolated from a rotted tree, Iwate Prefecture, Japan; CBS 8001 (NRRL Y-11996), type strain of *Candida cariosilignicola*, from decayed timber, Japan.

Type strain: CBS 7300.

Comments: Kumamoto et al. (1986) reported *P. methylivora* to ferment D-glucose, sucrose, trehalose and raffinose, but fermentation was not detected in the present study. Kurtzman and Robnett (unpublished) found *Candida cariosilignicola* and *P. methylivora* to have nearly identical nucleotide sequences in the variable 5′ region of the large subunit rRNA gene indicating the two taxa to be conspecific.

42.52. *Pichia mexicana* Miranda, Holzschu, Phaff & Starmer (1982)

Anamorph: *Candida terebra* Sugiyama & S. Goto

Synonyms:
Yamadazyma mexicana (Miranda, Holzschu, Phaff & Starmer) Billon-Grand (1989)
Candida terebra Sugiyama & S. Goto (1969)
Trichosporon veronae Florenzano (1953) nom. nud.
Candida veronae Florenzano ex van Uden & H.R. Buckley (1970)
Candida entomaea van der Walt, D.B. Scott & van der Klift (1972)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to cylindroidal, (1.1–3.4)×(1.9–9.0) µm, and occasionally tapered or curved. The cells occur singly, in pairs, or in small clusters. Growth is butyrous, dull-glistening and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows numerous well-branched pseudohyphae with moderately abundant blastoconidia. True hyphae were not detected, but some of the pseudohyphae show almost no constriction at the septa. Aerobic growth is white, butyrous, and with a dull surface; the colonies are striated and with an irregular margin. There is a faint ester-like odor.

Formation of ascospores: This species is heterothallic. Miranda et al. (1982) reported that following conjugation of complementary mating types the resulting zygote served as the ascus and that it is infrequent that diploid cells budded from the zygote undergo ascosporulation. Asci form one to four hat-shaped ascospores that are released at maturity. In the present study, conjugation was observed between complementary mating types, but ascospore formation was not detected. Miranda et al. (1982) noted that zygote formation and ascosporulation were infrequent events that most often occurred on 1–2% malt extract agar after 7–14 days.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/w	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	w/–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	s	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 7066 (Miranda et al. 1982).
Mol% G+C: 42.2–42.5, CBS 7066 and 4 additional strains (BD: Miranda et al. 1982).

Origin of the strains studied: CBS 7066 (UCD-FST 76-308A, NRRL Y-11818), from agria cactus (*Stenocereus gummosus*), Palmilla, Baja California Sur, Mexico; CBS 7067 (UCD-FST 76-391B, NRRL Y-11819), from organ-pipe cactus (*Stenocereus thurberi*), Rancho Cuñaño, Baja California Sur; CBS 6023 (NRRL Y-17683), type strain of *Candida terebra*, from soil, Japan; CBS 5815 (NRRL Y-17672), type strain of *Candida veronae*, from grape must, Italy; CBS 6306 (NRRL Y-7785), type strain of *Candida entomaea*, from an insect tunnel in pine (*Pinus radiata*), South Africa.

Complementary mating types: CBS 7066 (h^+) and CBS 7067 (h^-).

Type strain: CBS 7066.

Comments: *P. mexicana*, which is found in cacti from Baja California, Mexico, as well as from the southwestern U.S.A., has been isolated only as haploid mating types. Miranda et al. (1982) raised the possibility that *Candida terebra* and *C. entomaea* represent anamorphs of *P. mexicana*. C.-F. Lee et al. (1993) demonstrated through comparisons of nDNA complementarity that *C. terebra*, *C. entomaea* and *C. veronae* are conspecific. Kurtzman and Robnett (unpublished) found the nucleotide sequences from the 5′-end of the large subunit rRNA genes for all three *Candida* species, as well as for *P. mexicana*, to be identical, thus confirming *C. terebra*, the species of taxonomic priority, to represent the anamorph of *P. mexicana*.

42.53. *Pichia meyerae* van der Walt (1982a)

Anamorph: *Candida meyerae* van der Walt
Synonym:
Candida meyerae van der Walt (1982a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to cylindroidal, (1.5–3.6)×(1.6–6.2) µm, and occur singly, in pairs and in small clusters. Growth is butyrous, glistening and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows some well-branched pseudohyphae with blastoconidia. True hyphae were not observed, but van der Walt (1982a) reported their occurrence on corn meal agar. Aerobic growth is tannish-white, glistening, butyrous, and low convex with margins that are smooth to lobed. Odors produced by different strains range from faintly acid to faintly buttery.

Formation of ascospores: This species is heterothallic but it has been isolated from nature only in the diploid form. Asci from diploid strains are unconjugated and produce one to four hat-shaped ascospores that are released soon after formation. Van der Walt (1982a) obtained complementary mating types by heat treatment of an ascosporogenous culture of the type strain. Mixtures of complementary mating types are sexually agglutinative.

Ascosporulation was observed on 5% malt extract agar after 3 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 7076 (Billon-Grand 1985).
Mol% G+C: 48.5, 48.9, CBS 7077, 7078 (BD: Kurtzman, unpublished).

Origin of the strains studied: CBS 7076 (NRRL Y-17236), from rotting insect infested spurge (*Euphorbia ingens*), Groblersdal district, South Africa; CBS 7077 (NRRL Y-12777) and CBS 7078 (NRRL Y-12778), haploids from a heat-treated culture of CBS 7076.

Complementary mating types: CBS 7077 and CBS 7078, derived from CBS 7076.

Type strain: CBS 7076.

Comments: *P. meyerae* is phenotypically similar to *P. euphorbiae*, *P. rhodanensis*, *P. wickerhamii*, *P. veronae*, *P. amylophila* and *P. mississippiensis*, which are heterothallic as well. In pairings among these species, van der Walt and Opperman (1983) detected a mating reaction

only between *P. meyerae* and *P. euphorbiae*, but the resulting zygotes failed to produce ascospores.

42.54. *Pichia minuta* (Wickerham) Kurtzman (1984a)

This species has two varieties:

Pichia minuta (Wickerham) Kurtzman var. *minuta* (1984a)

Synonyms:

Hansenula minuta Wickerham (1951)
Zygohansenula minuta (Wickerham) Krasil'nikov (1954a)
Ogataea minuta (Wickerham) Y. Yamada, Maeda & Mikata (1994c)
?*Torulopsis methanolovescens* Oki & Kouno (Oki et al. 1972)
?*Candida methanolovescens* (Oki & Kouno) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Pichia lindneri Henninger & Windisch (1975a)

Pichia minuta var. *nonfermentans* (Wickerham) Kurtzman (1984a)

Synonyms:

Hansenula nonfermentans Wickerham (1969b)
Ogataea minuta (Wickerham) Y. Yamada, Maeda & Mikata var. *nonfermentans* (Wickerham) Y. Yamada, Maeda & Mikata (1994c)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, $(1.0–2.6)\times(1.0–3.2)\ \mu m$, and occur singly or in pairs.

Growth on the surface of assimilation media: Pellicles are not produced and generally there are no rings.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither hyphae nor pseudohyphae are detected under the coverglass. Aerobic growth is light tan, glistening, and butyrous to mucoid. Colony margins are entire. Some strains produce a faintly sweet odor.

Formation of ascospores: Ascospore formation is preceded by conjugation between a parent cell and a bud. Asci produce one to four hat-shaped ascospores and become deliquescent at maturity. The spores are quite small but increase in size following their release. Because of the mode of ascus formation and the observation that single-spore isolates from four-spored asci are sporogenous, the species is presumed to be homothallic (Kurtzman, unpublished). The variety *nonfermentans* is presumed to be homothallic for the same reasons.

Ascospores were observed for both varieties on 5% malt extract agar after 15–30 days at 25°C.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	w
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+
L-Sorbose	–	Ethanol	+/w
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	–	Citrate	s
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	v	Nitrate	v
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	v		

Co-Q: 7, CBS 1708, CBS 5764 (Yamada et al. 1973a).

Mol% G + C: 47.5, CBS 1708 (T_m: Nakase and Komagata 1971b); 46.7, 47.7, CBS 1708, 6502 (BD: Kurtzman 1984a), var. *minuta*; 45.6, CBS 5764 (T_m: Nakase and Komagata 1971b); 45.1, 45.3, CBS 5764 (BD: Kurtzman 1984a, Phaff et al. 1983), var. *nonfermentans*.

Supplementary description of *P. minuta* var. *nonfermentans*: The variety *nonfermentans* is capable of growth at 37°C and can be separated from the variety *minuta* on this characteristic. Additionally, the variety *nonfermentans* does not ferment glucose.

Origin of the strains belonging to the variety *minuta*: Isolated from fermenting mushrooms (*Mycena pura* Fries) by A.T. Henrici (1); the type strain of *P. lindneri*, NRRL Y-10948 (CBS 6502), forest soil, Germany.

Type strain: CBS 1708 (NRRL Y-411), from fermenting mushrooms.

Origin of the strain belonging to the variety *nonfermentans*: The only known strain of this variety was isolated from Libby Creek near Laramie, Wyoming, U.S.A.

Type strain: CBS 5764 (NRRL YB-2203).

Comments: The species described as *Hansenula minuta*, *H. nonfermentans* and *P. lindneri* have uniquely small cells and give similar reactions on fermentation and assimilation tests. Because of this phenotypic likeness, Kurtzman (1984a) compared the nDNA complementarity of the taxa and found 75% relatedness between *H. minuta* and *P. lindneri* and 48% relatedness between *H. minuta* and *H. nonfermentans*. These data indicate *H. minuta* and *P. lindneri* to be somewhat divergent strains of the same species. *H. nonfermentans* was regarded as a variety of *H. minuta*, but these two species may be sibling

species instead. As discussed under Comments on the genus, these data show that nitrate assimilation is not a reliable character for the separation of species or genera and prompted the transfer of *Hansenula* species with hat-shaped ascospores to *Pichia* which has taxonomic priority.

42.55. *Pichia mississippiensis* Kurtzman, Smiley, Johnson, Wickerham & Fuson (1980a)

Synonym:

Candida obtusa (Dietrichson) van Uden & do Carmo-Sousa ex van Uden & H.R. Buckley var. *arabinosa* Montrocher (1967)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells, which are quite variable in size, are ellipsoidal to elongate, or infrequently spheroidal, (1.1–5.0)×(2.0–12.0) μm, and occur singly, in pairs or in clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed but rings occasionally develop.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and highly branched pseudohyphae as well as infrequent true hyphae. True hyphae show no evidence of a dolipore septum when viewed under the light microscope. Aerobic growth is light tan, glistening, and butyrous; the center is raised and the margin entire. A faint ester-like odor is produced.

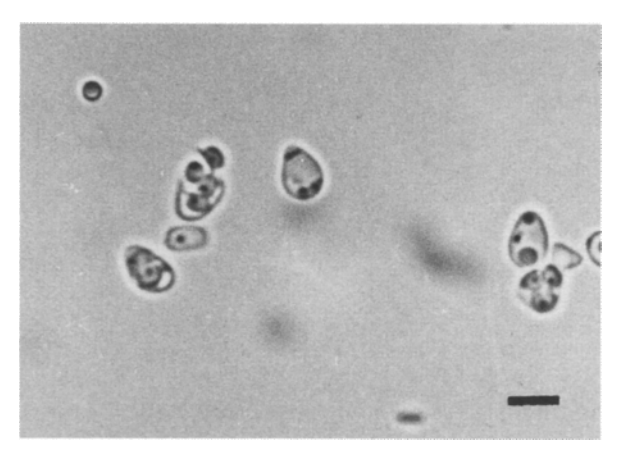


Fig. 145. *P. mississippiensis*, CBS 7023. Ascosporogenous culture after 3 days, 25°C, on 5% malt extract agar. Bar = 5 μm.

Formation of ascospores: Asci arise from diploid cells or from the pairing of complementary mating types and form two to four hat-shaped spores (Fig. 145). Asci are spheroidal to ellipsoidal and are free or attached to pseudohyphae. Infrequently, a pseudohyphal or hyphal cell may become an ascus. At maturity asci become deliquescent.

Ascospores were observed on 5% malt extract and YM agars after 5–8 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	w/–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 7023 (Billon-Grand 1985).

Mol% G + C: 47.2–48.0, CBS 7023 and three additional strains (BD: Kurtzman et al. 1980a).

Origin of the strains studied: Frass from shortleaf (*Pinus echinata*) and loblolly (*Pinus taeda*) pines, Mississippi, U.S.A. (9); laboratory contaminant (1); from a mushroom, France (1).

Complementary mating types: NRRL YB-1294-6 (CBS 7024) and NRRL YB-1294-7 (CBS 7025).

Type strain: CBS 7023 (NRRL YB-1294), from frass of a loblolly pine.

Comments: The relationship of this species with *P. amylophila* is discussed in the description of the latter species.

42.56. *Pichia naganishii* K. Kodama (Kodama & Kyono 1974a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (1.8–4.2)×(2.5–6.5) μm, and occur singly, in pairs or in small clusters. Growth is cream-colored, smooth, glistening and butyrous.

Growth on the surface of assimilation media: Thin rings are formed, but no pellicles.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is faint tan

in color, smooth, glistening and butyrous with an entire to finely lobed margin. A faint acidic odor is produced.

Formation of ascospores: Ascus formation may be preceded by conjugation between independent cells or between parent and bud. Two to four hat-shaped ascospores are produced in each ascus, and they are readily liberated after formation. Single-spore isolates from four-spored asci are sporogenous, indicating the species to be homothallic.

Ascospores were observed on 5% malt extract and YM agars after 3–5 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	+
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	w
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 6429 (Billon-Grand 1985).
Mol% G+C: 46.1, CBS 6429 (T_m: Kodama and Kyono 1974a).

Origin of the strain studied: Exudate from the plant *Camellia japonica* L., Japan (1).

Type strain: CBS 6429 (NRRL Y-7654).

Comments: The original description of *P. naganishii* (Kodama and Kyono 1974a) is generally in agreement with the results obtained in the present study. However, growth on L-sorbose, galactitol and citric acid proved to be negative rather than positive as originally reported.

42.57. *Pichia nakasei* J.A. Barnett, Payne & Yarrow (1983)

Anamorph: *Candida citrea* Nakase
Synonym:
Candida citrea Nakase (1971c)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to elongate, (1.6–4.6)×(2.1–8.6) µm, and single, in pairs, or small clusters. Growth is butyrous, dull-glistening and tannish-white.

Growth on the surface of assimilation media: Dry climbing pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant well-branched pseudohyphae with relatively few blastoconidia. True hyphae are not produced. Aerobic growth is white to tannish-white, dull-glistening, butyrous, and convex in profile with margins entire to lobed. A faintly acidic odor is produced.

Formation of ascospores: This species is heterothallic. Ascospores may be formed directly by diploid strains or following conjugation between complementary mating types. Asci produce one to four hat-shaped ascospores that are freed at maturity.

Ascospores were observed on 5% malt extract agar after 10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	w/–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+/w
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+/w		

Co-Q: Not determined.
Mol% G+C: 32.9, 33.2, CBS 6374 (AJ 4769), CBS 6737 (AJ 4441) (T_m: Nakase 1971a).

Origin of the strains studied: CBS 5141 (NRRL Y-7686), from apple must, Chile; CBS 6374 (NRRL Y-17248), the type strain of *Candida citrea,* from a lemon (*Citrus* sp.) fruit, Japan; CBS 6737 (NRRL Y-17237), from the fruit of a banana (*Musa sapientum*), Japan; CBS 6738 (NRRL Y-17238), from a lemon fruit, Japan.

Complementary mating types: CBS 6374 (*a*) and CBS 6737 (α).

Type strain: CBS 5141.

Comments: The type strain of *P. nakasei* maintained at CBS was initially an ascosporogenous diploid but reverted to a haploid mating type following long-term serial cultivation (Barnett et al. 1990), a phenomenon that has been observed for certain other heterothallic species (Wickerham et al. 1970a,b, Kurtzman and Smiley 1976). In contrast, the type strain maintained in the ARS Culture Collection (NRRL), which was obtained from CBS in 1974 and immediately lyophilized, is still ascosporogenous.

42.58. *Pichia nakazawae* K. Kodama (1975)

This species has two varieties:

Pichia nakazawae K. Kodama var. *nakazawae* (1975)

Synonym:
Yamadazyma nakazawae (K. Kodama) Billon-Grand (1989)

Pichia nakazawae var. *akitaensis* K. Kodama (1975)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.0–4.5)×(3.0–9.8) µm, and occur singly, in pairs and in clumps. Growth is cream-colored, smooth, glistening and butyrous.

Growth on the surface of assimilation media: Thin to moderately thick climbing pellicles are produced.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional branching outgrowths of ovoidal cells, but no pseudohyphae or true hyphae. Aerobic growth is tannish-white, smooth or finely striated, glistening, and butyrous with entire to finely lobed margins. A faintly acidic odor is produced.

Formation of ascospores: Ascus formation may be preceded by either conjugation between a parent cell and a bud or by conjugation between independent cells. Asci contain two to four hat-shaped ascospores and these are released soon after formation (Fig. 146). Both varieties of *P. nakazawae* were found to be homothallic through examination of single-spore isolates from four-spored asci (Kurtzman 1984b).

Ascospores for both varieties were observed on 5% malt extract and YM agars after 3–5 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	w		

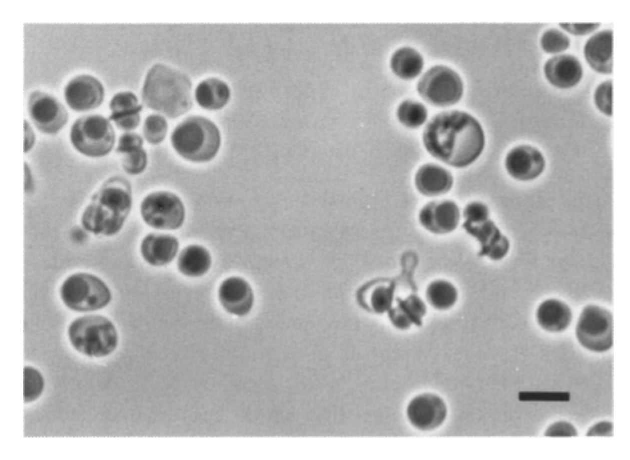


Fig. 146. *P. nakazawae* var. *nakazawae*, CBS 6700. Ascosporogenous culture after 2 weeks, 25°C, on 5% malt extract agar. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+/w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	s
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 6700 (Billon-Grand 1985).

Mol% G+C: 38.7 (T_m: Kodama 1975), 39.4 (BD: Kurtzman 1984b), CBS 6700, var. *nakazawae*; 39.5 (T_m: Kodama 1975), 39.9 (BD: Kurtzman 1984b), CBS 6701, var. *akitaensis*.

Supplementary description of *Pichia nakazawae* var. *akitaensis*: The variety *akitaensis* differs from the variety *nakazawae* by its failure to ferment galactose, by the presence of weak and slow fermentations of sucrose and trehalose, and from inability to assimilate L-rhamnose and lactic acid. The fermentation rate of variety *akitaensis* is quite weak in comparison to the vigorous ability of variety *nakazawae*. Another difference between the two varieties is the formation of moderately well-developed pseudohyphae by variety *akitaensis*, which causes agar-grown colonies to appear dull and finely convoluted.

Origin of the strain belonging to the variety *nakazawae*: Exudate of an oak (*Quercus myrsinaeforia* Blume), Japan (1).

Type strain: CBS 6700 (NRRL Y-7903).

Origin of the strain belonging to the variety *akitaensis*: Exudate of a willow (*Salix* sp.), Japan (1).

Type strain: CBS 6701 (NRRL Y-7904).

Comments: The original description of this species agrees with the results of the present study except that the variety *nakazawae* was found to slowly assimilate D-ribose and to give a weak and latent reaction on lactic acid. In addition, both varieties assimilated L-sorbose, but neither grew in vitamin-free medium. nDNA relatedness between the two varieties is 41%, suggesting that they are either divergent varieties of a single species or that they are sibling species. Varietal designations were maintained pending evidence that the pair is reproductively isolated (Kurtzman 1984b).

42.59. *Pichia norvegensis* Leask & Yarrow (1976)

Anamorph: *Candida norvegensis* Dietrichson ex van Uden & H.R. Buckley

Synonyms:

Candida zeylanoides (Castellani) Langeron & Guerra var. *norvegensis* Dietrichson (1954) nom. nud.

Candida norvegensis (Dietrichson) van Uden & Farinha (1958) nom. nud.

Candida norvegensis Dietrichson ex van Uden & H.R. Buckley (1970) (incorrectly described as *Candida norvegensis* (Dietrichson) van Uden & Farinha ex van Uden & H.R. Buckley)

Candida trigonopsoides Dietrichson (1954) nom. nud.

Candida mycoderma (Reess) Lodder & Kreger-van Rij var. *annulata* Dietrichson (1954) nom. nud.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, (1.7–4.1)×(3.0–10.0) µm, and occur singly, in pairs or in clusters. Growth is yellowish-white, smooth, glistening and butyrous.

Growth on the surface of assimilation media: Thin pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant pseudohyphae. Some of the longer pseudohyphal cells are markedly curved. True hyphae are not produced. Aerobic growth is light tan in color, smooth, glistening, butyrous and with an entire margin. A faint acidic odor is produced.

Formation of ascospores: Asci are unconjugated and form one to four hat-shaped ascospores which are released at maturity. Leask and Yarrow (1976) reported that heat-treatment of sporogenous cultures gave only sporogenous colonies, indicating the species to be homothallic.

Spores were observed on Fowell's acetate agar after 10–15 days at 25°C.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	w
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 6564 (Billon-Grand 1985).

Mol% G+C: 35.6, 36.6, IFO 1020, CBS 1922 (T_m: Nakase and Komagata 1971f), 35.5, 37.5, CBS 6564 (Billon-Grand 1981; BD: Kurtzman, unpublished).

Origin of the strain studied: Vagina of a pregnant woman (1).

Type strain: CBS 6564 (NRRL Y-7687).

Comments: Guého and Phaff (1983) demonstrated by nDNA reassociation that *Candida norvegensis* is the anamorph of *P. norvegensis*. These authors noted that in addition to its clinical association, *P. norvegensis* is commonly isolated from rots of opuntia cactus.

42.60. *Pichia ofunaensis* (Makiguchi & Y. Asai) Kurtzman (1996)

Synonym:

Hansenula ofunaensis Makiguchi & Y. Asai (Asai et al. 1976)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to elongate, (1.5–2.6)×(2.1–4.8) µm, and occasionally tapered or of irregular shapes. The cells occur singly, in pairs and in small clusters. Growth is butyrous, glistening and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows branched pseudohyphae with blastoconidia. True hyphae appear to be absent. Aerobic growth is butyrous, tannish-white, glistening and convex with a depressed center. Colony margins are entire to broadly lobed. A faint acidic odor is present.

Formation of ascospores: Ascospores were not observed in the present study. Asai et al. (1976) and Barnett et al. (1990) illustrated unconjugated asci containing one

to four hat-shaped ascospores. Asci become deliquescent. It is not known whether the species is homothallic or heterothallic.

Ascosporulation was reported to occur on YM agar after 7 days at room temperature.

Fermentation:

Glucose	ws	Lactose	–
Galactose	ws	Raffinose	–
Sucrose	–	Trehalose	ws
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	v
Cellobiose	s	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	s
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	s
D-Arabinose	–	Inositol	s
D-Ribose	s	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	ws	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	s		

Co-Q: Not determined.
Mol% G+C: 32.6, CBS 8129 (HPLC: Miller and Baharaeen 1979).

Origin of the strain studied: Soil, Ofuna, Japan.
Type strain: CBS 8129 (CBS 6861, NRRL Y-10998).

Comments: This species was described as nonfermentative, but a small amount of gas (2–5%) was found in Durham tube inserts after 12–16 days for glucose, galactose and trehalose. Assimilation reactions listed as variable did not repeatedly show growth on the designated compounds.

42.61. *Pichia ohmeri* (Etchells & T.A. Bell) Kreger-van Rij (1964b)

Synonyms:
Endomycopsis ohmeri Etchells & T.A. Bell (1950a)
Yamadazyma ohmeri (Etchells & T.A. Bell) Billon-Grand (1989)
Kodamaea ohmeri (Etchells & T.A. Bell) Y. Yamada, Suzuki, Matsuda & Mikata (1995)
Endomycopsis ohmeri Etchells & T.A. Bell var. *minor* Etchells & T.A. Bell (1950a)
Candida guilliermondii (Castellani) Langeron & Guerra var. *membranaefaciens* Lodder & Kreger-van Rij (1952)
Trichosporon arenicola de Lima & Aciole de Queiroz (1972)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to cylindroidal, (1.7–6.5)×(2.5–25) µm, and occur singly, in pairs and in short chains. Growth is somewhat pseudomycelial and cream-colored.

Growth on the surface of assimilation media: Pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, moderately branched pseudohyphae. True mycelium was not observed. Aerobic growth is grayish-white, dull and nearly smooth to finely convoluted. Margins are irregularly lobed.

Formation of ascospores: Infrequently, diploid strains are isolated which form unconjugated asci, but this heterothallic species is normally isolated in the haploid form and requires conjugation of complementary mating types before sporulation. Asci form one to four spores and are deliquescent at maturity. Spore shape is determined by the mating types and may be either hat-shaped or spheroidal (Wickerham and Burton 1954b). Pairing of NRRL Y-2078 (CBS 2037) and NRRL Y-2079 (CBS 568) gives hat-shaped spores while the conjugation of NRRL Y-2078 and NRRL Y-2080 (CBS 2038) produces spheroidal spores.

Ascospores were observed on 5% malt extract agar after 3–5 days at 25°C, following pairing of complementary mating types.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/w	Raffinose	+
Sucrose	+	Trehalose	w/–
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+
Inulin	v	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 5367 and 2 additional strains (Yamada et al. 1973a).
Mol% G+C: 42.2, 42.4, CBS 5367, AJ 5088 (T_m: Nakase and

Komagata 1970b); 44.3–44.7, CBS 5367 and 3 additional strains (BD: Fuson et al. 1980).

Origin of the strains studied: Cucumber brine (3); pleural fluid (1); gooseberry jelly (1); sambal ulak (1); flowers (2); unknown substrates (3).

Complementary mating types: NRRL Y-2078 (CBS 2037) and NRRL Y-2079 (CBS 568).

Type strain: CBS 5367 (NRRL Y-1932), from cucumber brine.

Comments: *Pichia ohmeri* is an unusual species because, depending upon the mating types paired, ascospores may be either hat-shaped or spheroidal (Wickerham and Burton 1954b). Preliminary genetic studies suggest that only a few genes determine which spore shape results (Kurtzman, unpublished). These studies also demonstrated ascospore fertility through the F_2 generation, thus indicating the various mating types to be members of the same species. This premise was confirmed by the work of Fuson et al. (1980), which showed the *P. ohmeri* mating types to exhibit 98–100% DNA relatedness.

42.62. *Pichia onychis* Yarrow (1965)

Synonym:
?*Pichia xylopsoci* van der Walt & D.B. Scott (1971c)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (2.0–4.5)×(2.8–7.8) µm, and occur singly or in pairs. Growth is cream-colored, glistening and butyrous.

Growth on the surface of assimilation media: Thin pellicles may be formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows a simple, sparingly branched pseudomycelium, but true hyphae are not formed. Aerobic growth is tannish-white, glistening, butyrous, and with an entire or occasionally lobed margin.

Formation of ascospores: Asci are unconjugated and produce one to four hat-shaped ascospores. The spores are liberated soon after formation. It has not been determined whether this species is homothallic or heterothallic.

Ascospores were observed on 5% malt extract, YM, and V8 agars after 10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	v	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	v		

Co-Q: 7 (Y. Yamada and H.J. Phaff, personal communication).
Mol% G+C: 40.8, 42.7, CBS 5587 (T_m: J.B. Fiol, cited by Billon-Grand 1981; BD: Kurtzman, unpublished).

Origin of the strains studied: Infected human nail (1); corn meal (1); dust (1).

Type strain: CBS 5587 (NRRL Y-7123), from an infected nail.

Comments: Results from the present study are in agreement with previous work (Yarrow 1965, Kreger-van Rij 1970c) except that growth on inulin was found to be positive for some strains. Yarrow (personal communication) identified the type strain of *P. xylopsoci* as *P. onychis*. Unfortunately, the culture was lost.

42.63. *Pichia opuntiae* Starmer, Phaff, Miranda, M.W. Miller & Barker (1979)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, (1.5–4.5)×(2.5–7.5) µm, and occur singly, in pairs or in short chains. Growth is butyrous and tannish-white.

Growth on the surface of assimilation media: Thin rings and incomplete pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows infrequent rudimentary pseudohyphae but no true hyphae. Aerobic growth is light tannish-white, smooth, glistening, butyrous, and low convex with entire to finely lobed margins. A faintly acidic odor is present.

Formation of ascospores: This species is heterothallic and haploid mating types are usually isolated from nature, but occasionally diploids are found. The ascospores, which are hat-shaped, usually form in diploid cells that bud from zygotes following mixture of complementary

mating types. There are commonly two ascospores per deliquescent ascus, but occasionally four are produced.

Ascospores were observed on YM agar at 25°C within 5–7 days following mixture of complementary mating types.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	v	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+/w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	s	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 7010 (Billon-Grand 1985).

Mol% G+C: 32.9–33.4, CBS 7010 and 5 additional strains (BD: Holzschu et al. 1985).

Origin of the strains studied: CBS 7010 (UCD-FST 77-40, NRRL Y-11707) and CBS 7011 (UCD-FST 77-42, NRRL Y-11708), both from rotted prickly pear cactus (*Opuntia inermis*), Yarrawonga, Australia.

Complementary mating types: CBS 7010 (h^+) and CBS 7011 (h^-).

Type strain: CBS 7010.

Comments: Results from the current study are in good agreement with the original description. The relationship of *P. opuntiae* with other cactophilic yeasts is discussed in the treatments of *P. antillensis* and *P. thermotolerans*. Separation of *P. opuntiae* from *P. antillensis* is unreliable when using results from standard assimilation tests, but it differs from *P. antillensis* by being killer negative. At present, *P. opuntiae* is known only from *Opuntia* cacti in Australia whereas *P. antillensis* appears restricted to columnar cacti of the West Indies.

42.64. *Pichia pastoris* (Guilliermond) Phaff (1956)

Synonyms:

Zygosaccharomyces pastori Guilliermond (1919)

Saccharomyces pastori (Guilliermond) Lodder & Kreger-van Rij (1952)

Petasospora pastori (Guilliermond) Boidin & Abadie (1954)

Zygowillia pastori (Guilliermond) Kudryavtsev (1960)

Zymopichia pastori (Guilliermond) Novák & Zsolt (1961)

Komagataella pastoris (Guilliermond) Y. Yamada, Matsuda, Maeda & Mikata (1995)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.0–4.0)×(2.2–5.8) μm, and occur singly or in pairs. Growth is cream-colored, dull to faintly glistening and butyrous.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is white, dull to faintly glistening and butyrous. Margins may be finely serrate or lobed.

Formation of ascospores: Asci may be unconjugated or show conjugation between parent cell and bud or between independent cells. One to four hat-shaped ascospores are formed in each ascus and they are soon liberated (Fig. 147). Single-spore isolates from four-spored asci are ascosporogenous, indicating this species to be homothallic.

Ascospores were observed on 5% malt extract, V8 and acetate agars after 10–20 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+/w
10% NaCl/5% glucose	v		

Co-Q: 8, CBS 704, IFO 948, IFO 1013 (Yamada et al. (1973a).

Mol% G+C: 40.2, 41.0, IFO 1013, CBS 704 (T_m: Nakase and Komagata 1970b); 42.8, CBS 704 (BD: Kurtzman, unpublished).

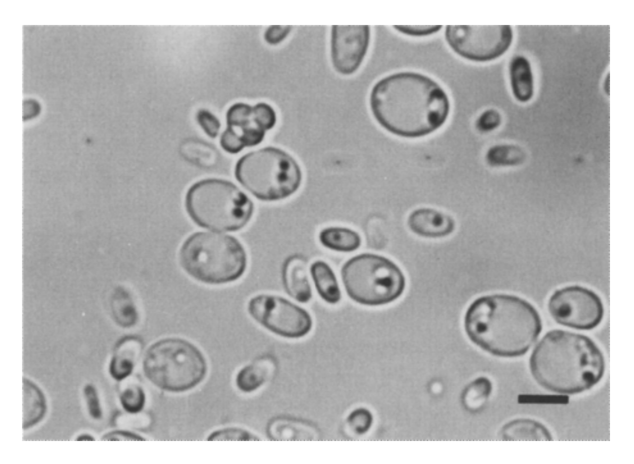


Fig. 147. *P. pastoris*, CBS 704. Ascosporogenous culture after 8 days, 25°C, on V8 agar. Bar = 5 μm.

Origin of the strains studied: Exudate of a chestnut tree (*Castanea* sp.), France (1); slime flux on oak (*Quercus kelloggii*), U.S.A. (1).

Type strain: CBS 704 (NRRL Y-1603), from a chestnut tree.

Comments: *P. pastoris* has become of considerable interest to biotechnologists because its gene expression system has been developed to produce large amounts of medically and industrially important proteins (Cregg et al. 1993). Two aspects of the species have contributed to its utility. First, fermentation techniques were developed for maintaining extremely high cell densities in excess of 100 g/l dry weight. Secondly, because *P. pastoris* assimilates methanol, the expression system is linked with alcohol oxidase which is abundantly produced in the presence of methanol.

42.65. *Pichia petersonii* (Wickerham) Kurtzman (1984a)

Synonym:
Hansenula petersonii Wickerham (1964b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ellipsoidal to elongate, (1.2–4.1)×(2.8–7.1) μm, and occur singly or in pairs. Growth is butyrous and light tan in color.

Growth on the surface of assimilation media: Smooth, mat pellicles and rings develop on certain media.

Dalmau plate culture on morphology agar: After 7 days at 25°C, abundant, highly branched pseudohyphae are formed under the coverglass. True hyphae are also produced by the mat oxidative forms of this species, but not by the smooth glistening types, such as NRRL YB-3807. Aerobic growth is light tan, smooth, dull to glistening, and butyrous. Margins of single colonies may be entire or lobed and surrounded by hyphal outgrowth.

Formation of ascospores: Vegetative cells directly convert to asci without prior conjugation. Asci form one to four hat-shaped ascospores which are freed through deliquescence of the ascus. Both heat-treated sporogenous cultures and single-spore isolates give rise to sporogenous colonies and the species is presumed to be homothallic. Wickerham (1964b) reported the presence of glistening colony forms, such as NRRL YB-3807, that were asporogenous.

Ascospores were observed on 5% malt extract and YM agars after 5–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 5555, CBS 5556 (Yamada et al. 1973a).
Mol% G + C: 43.9, 44.1, CBS 5556, CBS 5555 (T_m: Nakase and Komagata 1971b); 44.9, CBS 5555 (BD: Kurtzman et al. 1979).

Origin of the strains studied: Human cadavers preserved with embalming fluid (2).

Type strain: CBS 5555 (NRRL YB-3808).

Comments: Wickerham (1964b, 1970a) discussed the similarities of *P. petersonii*, *P. jadinii* and *C. utilis* and suggested that their primary habitat may be warm-blooded animals. All three species are resistant to toxic chemicals, especially embalming fluid, and van der Walt and Johannsen (1975b) observed a similar chemotolerance for *P. lynferdii*. As noted earlier, Kurtzman et al. (1979) demonstrated through nDNA reassociation studies that *P. jadinii* and *C. utilis* are conspecific whereas *P. petersonii* is a separate species.

42.66. *Pichia philodendri* (van der Walt & D.B. Scott) Kurtzman (1984a)

Synonyms:

Hansenula philodendri (as *H. philodendra*) van der Walt & D.B. Scott (Scott & van der Walt 1970b)

Ogataea philodendri (van der Walt & D.B. Scott) Y. Yamada, Maeda & Mikata (1994c)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.8–4.8)×(1.8–6.9) µm, and single, in pairs, or less frequently, in small clusters. Growth is butyrous and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither true hyphae nor pseudohyphae are detected under the coverglass. Aerobic growth is white, smooth to finely striated, glistening, and butyrous. Colony margins are usually lobed. A faintly acidic odor is present.

Formation of ascospores: Vegetative cells may convert directly to asci, or more frequently, ascus formation is preceded by conjugation between independent cells. Asci form one to four hat-shaped ascospores, and the spores are released through ascus deliquescence. Single-spore isolates from four-spored asci gave ascosporogenous colonies and the species is presumed to be homothallic (Kurtzman, unpublished).

Ascospores were observed on 5% malt extract, YM, and V8 agars after 3 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+
L-Sorbose	s	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	v	Citrate	s
D-Arabinose	–	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 6075 (Lee and Komagata 1980a).

Mol% G + C: 44.0, 49.7, CBS 6075 (HPLC: Miller and Baharaeen 1979; T_m: Lee and Komagata 1980a).

Origin of the strains studied: Frass from tunnels of the Bostrichid beetle (*Xylion adustus* Fahr.), which was infesting a moribund fig (*Ficus sycomorus* L.), near St. Lucia, Natal, South Africa (2).

Type strain: CBS 6075 (NRRL Y-7210).

42.67. *Pichia philogaea* van der Walt & E. Johannsen (1975c)

Synonym:

Yamadazyma philogaea (van der Walt & E. Johannsen) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal or cylindroidal, (2.8–7.5)×(3.0–10.0) µm, and occur singly, in pairs or in short chains. Growth is butyrous and cream-colored.

Growth on the surface of assimilation media: Thin pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows pseudohyphae to be common but relatively unbranched. True hyphae are not formed. Aerobic growth is tannish-white, glistening and smooth or finely striated. The margin is entire or occasionally lobed. A faintly acid odor is produced.

Formation of ascospores: Ascus formation is preceded by conjugation between a parent cell and a bud. Asci produce one to four hat-shaped ascospores which are released at maturity. Van der Walt and Johannsen (1975c) reported that heat-treated ascosporogenous cultures yielded only sporogenous isolates, and the species appears homothallic.

Ascospores were observed on 5% malt extract and YM agars after 3 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	w	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	w
10% NaCl/5% glucose	+		

Co-Q: 9, CBS 6696 (Billon-Grand 1985).
Mol% G+C: 42.0, CBS 6696 (T_m: Billon-Grand 1981).

Origin of the strain studied: Grassland soil, South Africa (2).

Type strain: CBS 6696 (NRRL Y-7813).

Comments: The results reported in the original description are generally in agreement with the findings of the present study. Van der Walt and Johannsen (1975c) noted sucrose fermentation to be slow but strong. It was negative in the present tests as was growth on salicin, originally reported as positive. *P. philogaea* shows considerable resemblance to *P. nakazawae* var. *nakazawae,* but differs by its inability to assimilate soluble starch, L-rhamnose and salicin.

42.68. *Pichia pijperi* van der Walt & Tscheuschner (1957a)

Synonym:
Hanseniaspora pijperi (van der Walt & Tscheuschner) Ditlevsen & Hjort (1964)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to cylindroidal, (3.0–5.8)×(5.0–20.0) µm, and occur singly, in pairs or in short chains. Growth is butyrous and cream-colored.

Growth on the surface of assimilation media: Dry, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows poorly to moderately well-developed pseudohyphae but no true hyphae. Aerobic growth is light tan, dull, generally smooth, and with an entire or finely lobed margin. A faint ester-like odor is produced.

Formation of ascospores: Asci are unconjugated and produce one to four hat-shaped ascospores. The spores are readily released at maturity. It is not known whether the species is homothallic or heterothallic.

Ascospores were observed on YM and 5% malt extract agars after 8–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 2887 (Yamada et al. 1973a).
Mol% G+C: 40.7, 42.5, CBS 2887 (T_m: Nakase and Komagata 1970b; BD: Fuson et al. 1980).

Origin of the strains studied: Buttermilk (1); *Drosophila melanogaster* (1).

Type strain: CBS 2887 (NRRL YB-4309), from buttermilk.

Comments: Despite the appearance of bipolar budding by *P. pijperi,* Kreger-van Rij (1970c) reported that bud scars observed under the transmission electron microscope were not the typical multiple scars of *Hanseniaspora,* but were like those seen in other species of *Pichia.*

42.69. *Pichia pini* (Holst) Phaff (1956)

Synonyms:
Zygosaccharomyces pini Holst (1936)
Petasospora pini (Holst) Boidin & Abadie (1954)
Saccharomyces pini (Holst) Shifrine & Phaff (1956)
Zygowillia pini (Holst) Kudryavtsev (1960)
Ogataea pini (Holst) Y. Yamada, Matsuda, Maeda & Mikata (1995)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (1.6–4.2)×(2.2–6.0) µm, and occur singly, in pairs and occasionally in small clusters. Growth is tannish-white, smooth, dull to glistening, and butyrous to somewhat mucoid.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows either no pseudomycelium or only a few poorly developed

pseudohyphae. True hyphae are not formed. Aerobic growth is tannish-white, smooth to finely striated, dull to glistening, and butyrous. Margins range from entire to lobed. A faint acidic odor is often produced.

Formation of ascospores: Asci may be unconjugated or show conjugation between a parent cell and a bud or between independent cells. Asci contain two to four hat-shaped ascospores which are liberated soon after formation. The presence of conjugation between cells and their buds suggests the species to be homothallic.

Ascospores were observed on 5% malt extract, V8 and corn meal agars after 2–10 days at 25°C.

Fermentation:

Glucose	v	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+/w
L-Sorbose	v	Ethanol	v
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	w/–
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	v
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	v		

Co-Q: 7, CBS 744 (Yamada et al. 1973a).

Mol% G + C: 44.4, 43.0, CBS 744 (T_m: Nakase and Komagata 1970b; BD: Fuson et al. 1980).

Origin of the strains studied: From the bark beetle *Dendroctonus brevicomis* (1), *D. pseudosugae* (2), *D. frontalis* (1), larvae of *D. monticolae* (1); Curculionidae larvae (1); *Hylugonotus brunneus* (1); *Araucaria araucana* (1); frass in shortleaf pine (*Pinus echinata*) (1); frass in loblolly pine (*Pinus taeda*) (1); frass from *Pinus* sp. (2); frass from pine (*Pinus contorta*) (4); frass from ponderosa pine (*Pinus ponderosa*) (1); frass from fir (*Abies firma*) (1); bark of a dead birch (*Betula* sp.) (1).

Type strain: CBS 744 (NRRL Y-11528), from *Dendroctonus brevicomis.*

Comments: As noted earlier, *P. pini* and *P. glucozyma* show great phenotypic similarity, and Kreger-van Rij (1964b) reported some strains of *P. pini* to assimilate nitrite. However, Kurtzman (unpublished) found the type strains of the two species to have only 3% nDNA relatedness.

42.70. *Pichia populi* (Phaff, Y. Yamada, Tredick & Miranda) Kurtzman (1984a)

Synonym:
Hansenula populi Phaff, Y. Yamada, Tredick & Miranda (1983)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal or occasionally elongate, (1.5–4.1)×(2.1–5.6) μm, and occur singly, in pairs and in small clusters. Growth is butyrous, semi-glistening and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows a few outgrowths of pseudohyphae comprised of undifferentiated cells. True hyphae are not formed. Aerobic growth is butyrous, tannish-white, glistening, and low convex with a raised center. Colony margins range from entire to lobed. A faintly acidic odor is present.

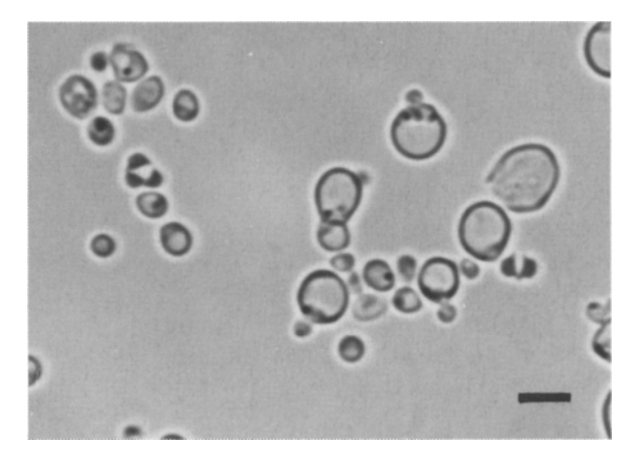


Fig. 148. *P. populi*, CBS 8094. Ascosporogenous culture after 2 weeks, 25°C, on 5% malt extract agar. Bar = 5 μm.

Formation of ascospores: This species is homothallic. Asci may arise from unconjugated cells or form after conjugation between independent cells or after conjugation between a cell and its bud. Asci produce one to four hat-shaped ascospores which are released by ascus deliquescence (Fig. 148).

Ascospores were observed on 5% malt extract agar after 7 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7 (Y. Yamada and H.J. Phaff, personal communication).
Mol% G+C: 43.8–44.2, CBS 8094 and 4 additional strains (BD: Phaff et al. 1983).

Origin of the strain studied: CBS 8094 (UCD-FST 68-628C, NRRL Y-12728) was isolated from the slime flux of a cottonwood tree (*Populus trichocarpa*) northwest of Dawson Creek, British Columbia, Canada.

Type strain: CBS 8094.

Comments: Phaff et al. (1983) noted *P. populi* to be phenotypically similar to *P. minuta* var. *nonfermentans* and *P. dryadoides*, but demonstrated no significant nDNA binding with either of these two taxa. Of the five strains of *P. populi* examined, all exhibited high nDNA relatedness except UCD-FST 68-603, which showed 77% complementarity with the type strain.

42.71. *Pichia pseudocactophila* Holzschu, Phaff, Tredick & Hedgecock (1983)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal to elongate, (1.6–5.0)×(1.9–6.6) µm, and single, in pairs and small clusters. Growth is tannish-white, dull-glistening and butyrous.

Growth on the surface of assimilation media: Dull, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows a few outgrowths of poorly differentiated pseudohyphae. True hyphae are not formed. Aerobic growth is white to tannish-white, glistening and butyrous with a lobed margin. In profile, colonies are low convex with a depressed center. A faint acidic odor is produced.

Formation of ascospores: This species is heterothallic. Newly isolated strains may be ascosporogenous diploids or haploid mating types. Asci form four hat-shaped ascospores that are liberated soon after formation.

Ascospore formation was observed on YM agar after 5–7 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 36.4–36.8, CBS 6929 and 5 additional strains (BD: Holzschu et al. 1983).

Origin of the strain studied: CBS 6929 (UCD-FST 77-427, NRRL Y-10966) was isolated from hecho cactus (*Pachycereus pecten-aboriginum*), Navojoa, Sonora, Mexico.

Complementary mating types: CBS 8095 (UCD-FST 77-427a, NRRL Y-17240) and CBS 8096 (UCD-FST 77-427b, NRRL Y-17723), derived from the type strain.

Type strain: CBS 6929.

Comments: Results from the present study are in good agreement with the original description. Holzschu et al. (1983) reported that most strains of *P. pseudocactophila* do not ferment glucose but a few of the isolates that they tested gave a late and weak fermentation of this sugar.

Strains of *P. pseudocactophila* were initially included among those described as *P. cactophila*. Isolates of *P. pseudocactophila* proved heterothallic and are noted for asci with four ascospores in contrast to the two-spored asci seen for *P. cactophila*. The two species show diminished nDNA relatedness (35%) and quite different allozyme profiles (Holzschu et al. 1983). Although *P. cactophila* is common to many species of cacti, *P. pseudocactophila* is generally recovered only from cardon cactus and its near

relatives. Shen and Lachance (1993) identified a strain of *P. cactophila* by restriction fragment mapping that was initially classified as *P. pseudocactophila* because it produced four-spored asci and was isolated from senita cactus. This report suggests that accurate identification of cactus yeasts will probably require molecular methods.

42.72. *Pichia quercuum* Phaff & Knapp (1956)

Synonym:
Zymopichia quercibus (Phaff & Knapp) Novák & Zsolt (1961)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (1.8–5.0)×(2.0–5.0) µm, and occur singly or in pairs. Growth is tannish-yellow, smooth, glistening and butyrous.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows very poorly differentiated pseudohyphae and no true hyphae. Aerobic growth is grayish-white, smooth, glistening, and butyrous with an entire to finely lobed margin. A faint acidic odor is present.

Formation of ascospores: Asci are generally conjugated with the pairing between parent cell and bud or between independent cells. One to four hat-shaped ascospores are produced in each ascus, and they are liberated soon after formation. The presence of conjugations between cells and their buds suggests the species is homothallic.

Ascospores were observed on 5% malt extract agar after 3 days at 25°C.

Fermentation:

Glucose	w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+/w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+/w
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 2283 (Yamada et al. 1973a).
Mol% G+C: 40.0, 42.7, CBS 2283 (T_m: Nakase and Komagata 1970b; BD: Kurtzman, unpublished).

Origin of the strain studied: From a slime flux of oak (*Quercus kelloggii*) (1).

Type strain: CBS 2283 (NRRL YB-4281).

42.73. *Pichia rabaulensis* Soneda & S. Uchida (1971)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2.0–5.2)×(3.0–9.0) µm, and occur singly, in pairs or occasionally in short chains. Growth is tannish-white, dull and finely wrinkled.

Growth on the surface of assimilation media: Heavy, dry, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant well-branched pseudohyphae, but relatively few blastoconidia are produced. True hyphae are not formed. Aerobic growth is tannish-white, dull, and almost powdery, and with fine striations. Margins may be entire, serrate or lobed. A faint ester-like odor is sometimes produced.

Formation of ascospores: Asci are unconjugated and produce one to four hat-shaped spores. The spores are liberated soon after formation. It is not known whether the species is homothallic or heterothallic.

Ascospores were observed on 5% malt extract and YM agars after 3–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w
Sucrose	+	Trehalose	w
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 6797 (Billon-Grand 1985).
Mol% G+C: 41.4, 44.2, CBS 6797 (T_m: Billon-Grand 1981; BD: Kurtzman, unpublished).

Origin of the strain studied: Feces of an African snail, Rabaul, New Britain, Papua New Guinea (1).

Type strain: CBS 6797 (NRRL Y-7945).

Comments: The present findings are in agreement with the original description of *P. rabaulensis*. Soneda and Uchida (1971) reported growth on galactose to be positive whereas the response to erythritol was variable. In the present study, growth on both of these compounds was negative.

42.74. *Pichia rhodanensis* (C. Ramírez & Boidin) Phaff (1956)

Synonyms:
Saccharomyces rhodanensis C. Ramírez & Boidin (1953a) (also in C. Ramírez & Boidin 1953b)
Petasospora rhodanensis (C. Ramírez & Boidin) Boidin & Abadie (1954)
Zymopichia rhodanensis (C. Ramírez & Boidin) Novák & Zsolt (1961)
Endomycopsis balearica Socias, C. Ramírez & Genestar (1954a)
Endomycopsis fibuligera (Lindner) Dekker var. *energica* Verona & Zardetto de Toledo (1959a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (2.6–6.0)×(4.0–10.0) µm, and occur singly, in pairs or in chains. Growth is light tan, dull or glistening, and smooth or wrinkled, depending upon the abundance of pseudohyphae.

Growth on the surface of assimilation media: Pellicles are sometimes present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant branched pseudohyphae with occasional verticillate clusters of blastoconidia. True hyphae are produced by some strains but not abundantly. Aerobic growth is light tan and may be dull or glistening and butyrous or somewhat tough and wrinkled, depending upon the abundance of hyphae and pseudohyphae. Colony margins are usually entire.

Formation of ascospores: This heterothallic species is frequently isolated from nature in the diploid form, and the unconjugated asci produce one to four hat-shaped ascospores. The spores are liberated soon after formation.

Ascospores were observed on 5% malt extract agar after 10–20 days.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	w/–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 5518 (Yamada et al. 1973a).
Mol% G+C: 50.2, 51.9, CBS 5518 (T_m: Nakase and Komagata 1970b; BD: Fuson et al. 1980, Kurtzman et al. 1980a).

Origin of the strains studied: Tanning liquor (4); stained leather (1); fermenting canaigre (1); palm nuts (1).

Complementary mating types: NRRL YB-651 sm-14 (CBS 4839) and NRRL Y-7854 Re-1 (CBS 7102).

Type strain: CBS 5518 (NRRL Y-7854), from tanning liquor.

Comments: As noted earlier, *P. rhodanensis* is phenotypically similar to *P. amylophila, P. euphorbiae, P. mississippiensis, P. meyerae, P. wickerhamii* and *P. veronae*, but none of these heterothallic species shows any mating response when paired with *P. rhodanensis*. Furthermore, *P. rhodanensis* shows little DNA relatedness with these other species (Kurtzman et al. 1980a).

42.75. *Pichia salicaria*[3] Phaff, M.W. Miller & Spencer (1964)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (1.7–4.5)×(2.3–7.2) µm, and occur singly or in pairs. Growth is tannish-yellow, smooth, dull-glistening and butyrous.

Growth on the surface of assimilation media: Heavy, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass has neither

[3] The original spelling of the species epithet *salictaria* has been treated as an orthographic error.

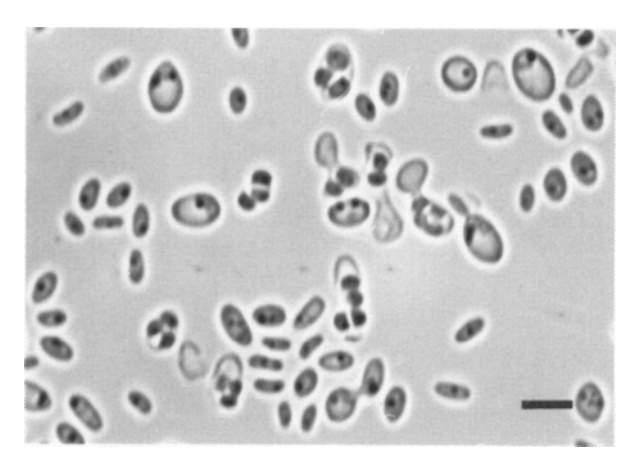


Fig. 149. *P. salicaria*, CBS 5456. Ascosporogenous culture after 3 days, 25°C, on 5% malt extract agar. Bar = 5 μm.

pseudohyphae nor true hyphae. Aerobic growth is tannish-white, smooth, dull-glistening, and butyrous. Margins are generally lobed. A faintly acidic odor is produced.

Formation of ascospores: Asci may be unconjugated or show conjugation between a parent cell and a bud, or between independent cells. One to four hat-shaped spores are formed per ascus and they are quickly liberated (Fig. 149). The presence of conjugation between cells and their buds suggests the species to be homothallic.

Ascospores were observed on 5% malt extract agar after 3 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 5456 (Yamada et al. 1973a).

Mol% G + C: 37.8, CBS 5456 (T_m: Nakase and Komagata 1970b).

Origin of the strains studied: Slime flux of willows (*Salix* sp.) (2); human toe (1).

Type strain: CBS 5456 (NRRL Y-6780).

42.76. *Pichia scolyti* (Phaff & Yoneyama) Kreger-van Rij (1964b)

Synonyms:
Endomycopsis scolyti Phaff & Yoneyama (1961)
Yamadazyma scolyti (Phaff & Yoneyama) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, (1.2–3.2) × (2.0–7.8) μm, and occur singly, in pairs and in chains. Growth is white to cream-colored, dull to glistening, and frequently finely wrinkled.

Growth on the surface of assimilation media: Rings and, occasionally, thin pellicles may form.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant moderately branched pseudohyphae with occasional dense clusters of blastoconidia. Some blastoconidia have an elongated triangular appearance. True hyphae may also be present. Aerobic growth is white to tannish-white, dull or glistening, frequently finely wrinkled, and occasionally papillate. Margins are irregularly serrate.

Formation of ascospores: This species is heterothallic and usually isolated from nature in the haploid form, although at least one diploid has been recovered as a natural isolate. Many of the haploids show little mating and such pairs form few ascospores. Asci produce one to four hat-shaped ascospores which are liberated soon after formation.

Ascospores were observed on 5% malt extract agar after 2–20 days at 25°C.

Fermentation:

Glucose	ws	Lactose	–
Galactose	ws	Raffinose	–
Sucrose	w/–	Trehalose	ws
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	s
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	v		

Co-Q: 9, CBS 4802 and 3 additional strains (Yamada et al. 1973a).
Mol% G+C: 36.3, 40.1, CBS 4802 (T_m: J.B. Fiol cited by Billon-Grand 1981; BD: Fuson et al. 1980).

Origin of the strains studied: From frass of the bark beetle *Scolytus ventralis* in fir (*Abies concolor*), California (3); frass in Balsam fir (*Abies balsamea*), Ontario, Canada (1); from Douglas fir beetle (*Dendroctonus pseudotsugae*), Washington (2).

Complementary mating types: NRRL Y-5512 (CBS 4802) and NRRL Y-5513 (CBS 4803), from Phaff and Yoneyama (1961); NRRL YB-1403-1 (CBS 5731) and NRRL YB-1403-2 (CBS 5732), isolated by Wickerham from frass obtained in Ontario, Canada. These latter haploids seem to have greater mating competence.

Type strain: CBS 4802 (NRRL Y-5512), from frass of *Scolytus ventralis.*

42.77. *Pichia segobiensis* Santa María & García Aser (1977)

Synonym:
Yamadazyma segobiensis (Santa María & García Aser) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.0–4.5)×(2.5–6.3) μm, and occur singly and in pairs. Growth is tannish-white, smooth, glistening, and butyrous.

Growth on the surface of assimilation media: Rings and, occasionally, thin pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows infrequent and poorly differentiated pseudohyphae. True hyphae are not formed. Aerobic growth is light tannish-white, smooth to occasionally slightly papillate, moderately glistening, butyrous, and with an entire to sparingly lobed margin. A faint acidic odor is formed.

Formation of ascospores: Conjugation between a parent cell and a bud or between independent cells may precede ascus formation. One, but usually two hat-shaped ascospores are formed in each ascus, and they are readily liberated. The mode of conjugation suggests the species to be homothallic.

Ascospores were observed on 5% malt extract agar after 3–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 9, CBS 6857 (Billon-Grand 1985).
Mol% G+C: 41.0, 42.0, CBS 6857 (T_m: Billon-Grand 1981; BD: Kurtzman, unpublished).

Origin of the strains studied: From an adult larva of the insect *Calcophora mariana massiliensis* in Scotch pine (*Pinus silvestris*) (1); insect frass in the bark of *P. silvestris* (1); larva of the long-horned beetle *Rhagium inquisitor* (1).

Type strain: CBS 6857 (NRRL Y-11571), from a larva of *Calcophora mariana massiliensis.*

Comments: Results from the present study are in good agreement with the original description of *P. segobiensis.* The only difference noted was that Santa María and García Aser (1977) reported growth on D-arabinose to be negative, whereas it was slow but positive in the current examination.

P. segobiensis, P. stipitis, Candida shehatae and *Pachysolen tannophilus* are of interest to biotechnologists because of their ability to fermentatively produce ethanol from D-xylose, a major pentose in plant biomass (Toivola et al. 1984). With the exception of *P. segobiensis,* the phylogenetic relationships among xylose-fermenting species are discussed under *P. stipitis*. More recently, comparisons of nDNA complementarity have shown ca. 40% relatedness between *P. segobiensis* and *P. stipitis,* demonstrating the two taxa to be sibling species (Kurtzman and Robnett, unpublished).

42.78. *Pichia silvicola* (Wickerham) Kurtzman (1984a)

Synonyms:
Hansenula silvicola Wickerham (1951)
Endomycopsis muscicola Nakase & Komagata (1966)
Hansenula muscicola (Nakase & Komagata) Yarrow (1972)
Pichia muscicola (Nakase & Komagata) Kurtzman (1984a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ellipsoidal to elongate, (1.0–4.0)×(1.2–9.5) μm and occur singly, in pairs, or in small clusters. Growth is butyrous to mycelial and tannish-white in color.

Growth on the surface of assimilation media: Exceptionally thin pellicles are formed by some isolates and thin rings may be produced.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and highly branched pseudohyphae. True hyphae are occasionally detected. Aerobic growth is white to tannish-white, smooth, glistening or dull, and butyrous. Colony margins are entire or occasionally lobed and may show hyphal outgrowths. An ester-like odor is usually present.

Formation of ascospores: Asci may be unconjugated or show conjugation between parent and bud, or between independent cells. Asci form one to four hat-shaped ascospores that are freed when the ascus deliquesces (Fig. 150). Single-spore isolates obtained from four-spored asci of the type strain formed ascosporogenous colonies, and the species is presumed to be homothallic (Kurtzman, unpublished).

Ascospores were observed on 5% malt extract agar after 5–20 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/w	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 1705, CBS 5800, and 3 additional strains (Yamada et al. 1973a).

Mol% G+C: 34.1–35.1, CBS 1705 and 3 additional strains (T_m: Nakase and Komagata 1971b); 36.5, CBS 1705 (BD: Kurtzman, unpublished).

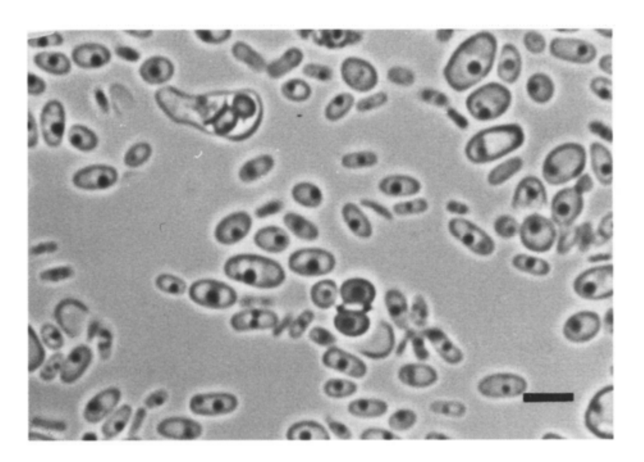


Fig. 150. *P. silvicola*, CBS 1705. Ascosporogenous culture after 8 days, 25°C, on 5% malt extract agar. Bar = 5 μm.

Origin of the strains studied: Gums from wild black cherry trees (*Prunus serotina* Ehrh.), Peoria, Illinois, U.S.A. (3); gums from wild pin cherry trees (*Prunus pensylvanica* Linn.), Wisconsin and Vermont, U.S.A. (4); the type strain of *P. muscicola* (CBS 5800, NRRL Y-7005) and two additional strains of this taxon, from moss, Tottori Prefecture, Japan.

Type strain: CBS 1705 (NRRL Y-1678), isolated from gum of *P. serotina,* Peoria, Illinois.

Comments: In a comparison of sequences from the 5′-end of large subunit rRNAs, Kurtzman and Robnett (manuscript in preparation) noticed that *P. silvicola* and *P. muscicola* differed by just one nucleotide in this variable region. On the basis of earlier work, this result indicates that *P. muscicola* and *P. silvicola* are conspecific.

42.79. *Pichia spartinae* Ahearn, Yarrow & Meyers (1970)

Synonym:

Yamadazyma spartinae (Ahearn, Yarrow & Meyers) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.5–5.3)×(3.3–6.5) μm, and occur singly, in pairs or occasionally in short chains. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin rings are formed and occasionally pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only occasional pseudohyphae, but these are moderately branched and may bear blastoconidia. True hyphae are not formed. Aerobic growth is light tan, smooth, glistening and butyrous. Margins are entire to lobed.

Formation of ascospores: Asci are unconjugated or conjugated if formed from the pairing of complementary mating types. Occasionally one, but usually, two, hat-shaped ascospores form in one of the conjugants while the other remains empty. Asci become deliquescent at maturity. The type strain, NRRL Y-7322 (CBS 6059), is

now asporogenous, but conjugates and sporulates when paired with mating type NRRL Y-7665-3.

Ascospores were observed on 5% malt extract agar after 5–7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	w/–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+		

Co-Q: 9 (Billon-Grand 1989).
Mol% G+C: 38.0, 41.1, CBS 6059 (T_m: Billon-Grand 1981; BD: Kurtzman, unpublished).

Origin of the strains studied: From oyster grass (*Spartina alterniflora* Loisel) marshes, Barataria Bay, Louisiana, U.S.A. (7).

Complementary mating types: NRRL Y-7665-1 (CBS 6666) and NRRL Y-7665-3 (CBS 6669).

Type strain: CBS 6059 (NRRL Y-7322).

Comments: In the original description, ascospores of *P. spartinae* were reported to be spheroidal or infrequently oval with a slight ledge (Ahearn et al. 1970). Later, Kurtzman and Ahearn (1976) showed a high incidence of malformed spores among strains of *P. spartinae*, and that mature spores were hat-shaped rather than spheroidal.

Pichia spartinae is phenotypically similar to several other heterothallic species of *Pichia* and to certain species of *Candida*. However, there was no mating response between *P. spartinae* and *P. angophorae, P. rhodanensis, P. wickerhamii, P. veronae, Candida freyschussii, C. maritima, C. obtusa,* or *C. oregonensis* (Kurtzman and Ahearn 1976).

42.80. *Pichia stipitis* Pignal (1967)

Synonym:
Yamadazyma stipitis (Pignal) Billon-Grand (1989)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.2–4.5)×(2.5–6.0) µm, and occur singly or in pairs. Growth is cream-colored, dull-glistening, smooth to faintly wrinkled and butyrous.

Growth on the surface of assimilation media: Rings and incomplete pellicles may form.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and well-branched pseudohyphae with occasional blastoconidia. True hyphae are not formed. Aerobic growth is light tan, smooth to finely wrinkled, dull-glistening, butyrous, and with an entire to lobed margin. A faintly acidic odor is often present.

Formation of ascospores: Preceding ascus formation, there may be conjugation between a parent cell and a bud or between independent cells, or the ascus may be unconjugated but with a protuberance. Asci produce two hat-shaped ascospores that are released soon after formation (Fig. 151). Single-spore isolates produce ascosporogenous colonies, but because only two ascospores are formed in each ascus, it is not certain whether meiosis has occurred (Kurtzman 1990b). The presence of parent cell–bud conjugation suggests the species is homothallic.

Ascospores were observed on 5% malt extract agar after 3–5 days at 25°C.

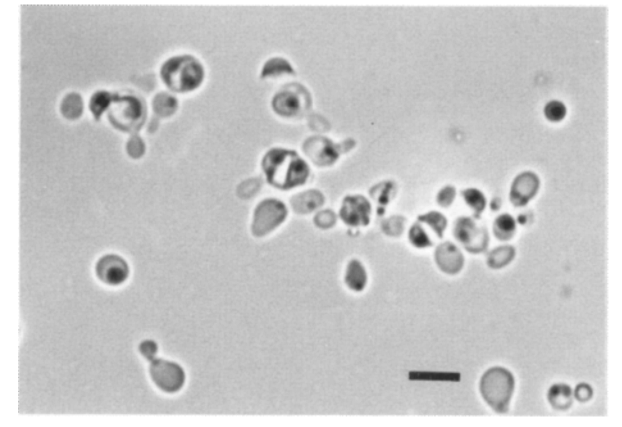


Fig. 151. *P. stipitis*, CBS 5773. Ascosporogenous culture after 1 week, 25°C, on 5% malt extract agar. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	w	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	v		

Co-Q: 9, CBS 5773 (Billon-Grand 1989).

Mol% G+C: 42.6, CBS 5773 (BD: Miranda et al. 1982); 43.2–43.6, CBS 5773 and 4 additional strains (BD: Kurtzman 1990b).

Origin of the strains studied: From larvae of the insects *Cetonia* sp., *Dorcus parallelipipedus* and *Laphria* (4); from J. Santa María (1).

Type strain: CBS 5773 (NRRL Y-7124), from insect larvae.

Comments: *P. stipitis, P. segobiensis, Candida shehatae* and *Pachysolen tannophilus* are of industrial interest because they ferment D-xylose to ethanol. On the basis of phenotypic similarity, Kreger-van Rij (1970c) suggested that *P. stipitis* and *C. shehatae* may represent teleomorphic and anamorphic states of the same species. Vaughan-Martini (1984), however, found that there was little nDNA relatedness between type strains of the two taxa. Kurtzman (1990b) showed from rRNA sequence comparisons that *P. stipitis* and *C. shehatae* are closely related but separate species. In contrast, *P. tannophilus* is more distantly related.

42.81. *Pichia strasburgensis* (C. Ramírez & Boidin) Phaff (1956)

Synonyms:

Saccharomyces strasburgensis C. Ramírez & Boidin (1953a) (also in C. Ramirez & Boidin 1953b)

Petasospora strasburgensis (C. Ramírez & Boidin) Boidin & Abadie (1954)

Zymopichia strasburgensis (C. Ramírez & Boidin) Novák & Zsolt (1961)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, (1.5–4.5)×(2.9–14.0) µm, and occur singly or in pairs. Growth is cream-colored, smooth, faintly glistening and butyrous.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows pseudohyphae but no true hyphae. Some strains produce only occasional, sparingly branched pseudohyphae while others form considerable pseudomycelium that may be well-branched and bear clusters of blastoconidia. Aerobic growth is pale tannish-white, smooth to finely wrinkled, dull-glistening and butyrous. Margins may be serrate or lobed. A faint ester-like odor is often present.

Formation of ascospores: Asci form without prior conjugation and contain one to four hat-shaped ascospores which are readily liberated. Kreger-van Rij (1964b) reported this species to be heterothallic.

Ascospores were observed on 5% malt extract agar after 3 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/w	Raffinose	w
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 2939 (Yamada et al. 1973a).

Mol% G+C: 40.2, 40.6, CBS 2039 (T_m: Nakase and Komagata 1970b; BD: Fuson et al. 1980).

Origin of the strains studied: Stained leather, from Ramírez (2) and from Boidin (1).

Complementary mating types: CBS 2039 (NRRL Y-11980) and CBS 5005 (NRRL Y-11981).

Type strain: CBS 2939 (NRRL Y-2383), from stained leather.

42.82. *Pichia subpelliculosa* Kurtzman (1984a)

Synonyms:
Hansenula subpelliculosa Bedford (1942) nom. nud.
Endomycopsis subpelliculosa (Bedford) Novák & Zsolt (1961) nom. nud.
Hansenula anomala (E.C. Hansen) H. & P. Sydow var. *subpelliculosa* K. Kodama, Kyono & S. Kodama (1957) nom. nud.
Hansenula subpelliculosa Bedford ex J.A. Barnett, Payne & Yarrow (1983)
Pseudohansenula peiping Mogi (1942) nom. nud.
Hansenula arabitolgenes Fang nom. nud.? (Chang et al. 1963)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ellipsoidal, (1.9–5.1)×(3.4–9.2) µm, and occur singly or in pairs. Growth is white to tannish-white and butyrous.

Growth on the surface of assimilation media: Thin pellicles are occasionally formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass usually shows abundant and highly branched pseudohyphae, although some strains fail to produce any pseudohyphae. True hyphae are formed, but they are not common. Aerobic growth is white to tannish-white, smooth, glistening, and butyrous. Colony margins are usually lobed and devoid of pseudohyphal outgrowths. A faint, pleasant odor may be detected.

Formation of ascospores: Asci are unconjugated and form one to four hat-shaped ascospores. The asci become deliquescent at maturity. Wickerham and Burton (1954a) demonstrated this species to be heterothallic by heat-treatment of sporogenous cultures.

Ascospores were observed on 5% malt extract and V8 agars after 5–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	w/–
Sucrose	+	Trehalose	–
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	+		

Co-Q: 7, CBS 5767 and 3 additional strains (Yamada et al. 1973a).
Mol% G+C: 33.4–33.9, AJ 5343, AJ 5344, AJ 5345 (T_m: Nakase and Komagata 1971b); 34.0, CBS 5767 (BD: Kurtzman, unpublished).

Origin of the strains studied: Bedford's strains from sugar-egg concentrate (1), syrup (1), grape juice (1), and dried prunes (1); Etchell's strains from fermenting cucumbers and from brined unshelled peas and green beans (27); soy sauce manufacturing, China (1); miso (1); NRRL YB-3328, *Pseudohansenula peiping*; CBS 7164 (NRRL Y-17244), *Hansenula arabitolgenes.*

Complementary mating types: NRRL Y-1683-11 (CBS 1997) and NRRL Y-1822-12 (CBS 1996).

Type strain: CBS 5767 (NRRL Y-1683), from fermenting cucumbers.

Comments: *Hansenula subpelliculosa* was described by Bedford (1942) without benefit of a Latin diagnosis or the designation of a type strain. In the transfer of hat-spored species of *Hansenula* to *Pichia*, Kurtzman (1984a) provided the requisite Latin description and designated as type strain an isolate from fermenting cucumber brines that Wickerham (1951) found to be typical of the species. Barnett et al. (1983) also provided a Latin description of this species, but their validation was for *Hansenula subpelliculosa* and would require further emendation if it were to be used.

P. subpelliculosa, P. anomala and *P. fabianii* are quite similar when compared by the standard tests. *P. anomala* is separated from the other two species by growth in vitamin-free medium, and *P. subpelliculosa* is separated from *P. fabianii* by ability to assimilate erythritol. Wickerham and Burton (1954a) indicated that these three heterothallic taxa do not interbreed.

Kurtzman and Robnett (manuscript in preparation) compared the 5′-end sequences of large subunit rRNAs for all known species of *Pichia. P. subpelliculosa* and *Hansenula arabitolgenes* have identical sequences suggesting that they are conspecific. *H. arabitolgenes* appears to have been published without a Latin description.

42.83. *Pichia sydowiorum* (D.B. Scott & van der Walt) Kurtzman (1984a)

Anamorph: *Candida nitrativorans* van der Walt, D.B. Scott & van der Klift

Synonyms:
Hansenula sydowiorum D.B. Scott & van der Walt (1970a)
Candida nitrativorans van der Walt, D.B. Scott & van der Klift (1972)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.8–5.3)×(2.7–7.8) µm, and occur singly or in pairs. Growth is butyrous and light tannish-white in color.

Growth on the surface of assimilation media: Pellicles form and are smooth and waxy.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant and well-branched pseudohyphae. True hyphae are not produced. Aerobic growth is white, smooth, dull to moderately glistening, and butyrous. Colony margins are generally lobed.

Formation of ascospores: Asci are unconjugated. Scott and van der Walt (1970a) reported vegetative cells to first become lipid-rich and thick-walled, and following a dormant period, an outer membrane is shed to form the ascus. One to four hat-shaped ascospores are formed per ascus, and asci are deliquescent. Heat-treatment of sporogenous cultures gave only sporogenous colonies, and the species is presumed to be homothallic (Scott and van der Walt 1970a).

Ascospores were observed on YM and 5% malt extract agars after 10 days at 15°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/w	Raffinose	w
Sucrose	+	Trehalose	s
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: Not determined.
Mol% G + C: 36.2, CBS 5995 (HPLC: Miller and Baharaeen 1979).

Origin of the strains studied: Frass from tunnels of the Bostrichid beetle (*Sinonylon ruficorne* Fahr.), infesting *Combretum apiculatum* Sond. (3); frass from tunnels in *Mimusops caffra* (1), all from South Africa.

Type strain: CBS 5995 (NRRL Y-7130), from tunnels in *S. ruficorne*.

Comments: *P. sydowiorum* is similar to *P. ciferrii* and *P. anomala* but the three species can be separated by their assimilation reactions on L-rhamnose and melibiose. As noted earlier, *P. ciferrii* forms crystals of tetraacetyl-phytosphingosine whereas *P. sydowiorum* synthesizes 2-D-hydroxyhexadecanoic acid (Vesonder et al. 1970). Crystalline compounds have not been observed in cultures of *P. anomala*.

In contrast to the original description which reported the assimilation of soluble starch and D-xylose to be positive, two of the four strains of *P. sydowiorum* examined in the present study gave negative reactions on these compounds. In addition, two of the strains failed to ferment maltose.

42.84. *Pichia tannicola* F.H. Jacob (1969a)

Synonym:
Pichia abadieae F.H. Jacob (1969b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.2–4.1)×(2.0–5.4) µm, and occur singly or in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional, sparingly branched pseudohyphae, but no true hyphae. Aerobic growth is light tannish-white in color, smooth, glistening and butyrous with a finely serrate margin.

Formation of ascospores: Ascospores were not observed in the present study. Jacob (1969a,b) reported the asci, which became deliquescent, to produce one to four subspheroidal ascospores devoid of surface ornamentation. Asci were unconjugated or showed conjugation between a cell and its bud.

Ascospores were observed on sterilized carrot slices (Jacob 1969a,b).

Fermentation:

Glucose	+/w	Lactose	–
Galactose	ws	Raffinose	–
Sucrose	–	Trehalose	+/w
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	w/–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	s
Lactose	v	D-Mannitol	s
Melibiose	+	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	w/–
Soluble starch	–	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+/w
D-Arabinose	–	Inositol	v
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G+C: 40.7, CBS 6065 (T_m: J.B. Fiol cited by Billon-Grand 1981); 46.0, 43.0, CBS 6067 (T_m: Fiol and Billon-Grand 1981; BD: Kurtzman, unpublished).

Origin of the strains studied: CBS 6067 (NRRL Y-7499), type strain, *P. abadieae*; CBS 6065 (NRRL Y-17392), type strain, *P. tannicola*; both from tanning fluid, France.

Type strain: CBS 6065.

Comments: *P. abadieae* and *P. tannicola* were separated from one another because of differences in their assimilation of lactose, erythritol, D-gluconate and inositol. However, Kurtzman and Robnett (unpublished) determined that type strains of the two species showed 100% nDNA complementarity. Because of taxonomic priority, *P. abadieae* is a synonym of *P. tannicola.*

42.85. *Pichia thermotolerans* (Starmer, Phaff, Miranda, M.W. Miller & Barker) Holzschu, Phaff, Tredick & Hedgecock (1985)

Synonym:

Pichia opuntiae Starmer, Phaff, Miranda, M.W. Miller & Barker var. *thermotolerans* Starmer, Phaff, Miranda, M.W. Miller & Barker (1979)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, (1.5–4.5)×(2.5–7.5) µm, and occur singly, in pairs or in short chains. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Thin rings and incomplete pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows infrequent rudimentary pseudohyphae but no true hyphae. Aerobic growth is light tannish-white, smooth, glistening, butyrous, and low convex with entire to finely lobed margins. A faintly acidic odor is present.

Formation of ascospores: This species is heterothallic, and the majority of strains isolated from nature are asporogenous haploids. Following the pairing of complementary mating types, zygotes usually bud diploid cells which become asci. Each ascus contains two to four hat-shaped ascospores which are liberated soon after formation. Ascospores were not seen in the present study, and Starmer et al. (1979) commented that formation of asci is extremely rare.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G+C: 32.8–33.0, CBS 7012 and 5 additional strains (BD: Holzschu et al. 1985).

Origin of the strains studied: CBS 7012 (UCD-FST 76-211, NRRL Y-11709), from senita cactus (*Lophocereus schottii*), Rancho Cuñaño, Baja California Sur, Mexico; CBS 7013 (UCD-FST 76-385B, NRRL Y-11710), from soil under cardon cactus (*Pachycereus pringlei*), Mulegé, Baja California Sur, Mexico.

Complementary mating types: CBS 7012 (h^+) and CBS 7013 (h^-).

Type strain: CBS 7012.

Comments: *P. thermotolerans* was initially described as a variety of *P. opuntiae* because some mating was shown between the two taxa (Starmer et al. 1979). Holzschu et al. (1985) reexamined the relationship, noting that intervarietal matings produced only a few ascospores of unknown viability, that the varieties averaged 28% nDNA complementarity, and that allozyme patterns suggested the two varieties to be genetically isolated. From these data, the variety *thermotolerans* was elevated to species status.

P. antillensis is closely related to *P. opuntiae* and *P. thermotolerans,* showing 50% and 26% nDNA complementarity, respectively, to the two species (Starmer et al. 1984). However, *P. antillensis* exhibited no mating response when paired with either *P. opuntiae* or *P. thermotolerans*. Lachance et al. (1988) and Starmer et al. (1990) reviewed the genetics and biogeography of the cactus yeasts.

42.86. *Pichia toletana* (Socias, C. Ramírez & Peláez) Kreger-van Rij (1964b)

Synonyms:

Debaryomyces toletanus Socias, C. Ramírez & Peláez (1954b)

Zymodebaryomyces toletanus (Socias, C. Ramírez & Peláez) Novák & Zsolt (1961)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (1.5–5.0)×(3.0–9.7) µm, and occur singly and in pairs. Growth is yellowish-white, smooth to wrinkled and dull-glistening.

Growth on the surface of assimilation media: Thin pellicles are formed by some strains.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, branched pseudohyphae with blastoconidia in a verticillate arrangement. True hyphae are not formed. Aerobic growth is tannish-white, smooth to wrinkled, dull-glistening and butyrous. Margins are usually lobed.

Formation of ascospores: Conjugation of a parent cell and a bud usually precedes ascus formation. Asci contain one to four hat-shaped ascospores which may be quickly or slowly liberated. Single-spore isolates from four-spored asci are sporogenous, indicating the species to be homothallic.

Ascospores were observed on 5% malt extract agar after 2–7 days at 25°C.

Fermentation:

Glucose	ws	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+/w
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	+/w
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	ws
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 2504 (Yamada et al. 1973a).
Mol% G+C: 39.3, 39.6, CBS 2504 (T_m: Nakase and Komagata 1970b; BD: Kurtzman 1987a).

Origin of the strains studied: Tanning fluid (1); *Drosophila miranda* (1); frass of dead pine (*Pinus* sp.) (1); diseased fir (*Abies nobilis*) (1).

Type strain: CBS 2504 (NRRL YB-4247), from tanning fluid.

42.87. *Pichia trehalophila* Phaff, M.W. Miller & Spencer (1964)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.1–6.8)×(3.5–7.5) µm, and occur singly and in pairs. Growth is tannish-yellow, smooth, glistening and butyrous.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is tannish-white, smooth, glistening and butyrous. Margins are entire.

Formation of ascospores: Ascus formation may be preceded by conjugation between a parent cell and a bud or between independent cells. Cells with protuberances are also found in sporulating cultures. Two to four hat-shaped ascospores are formed per ascus and they are quickly liberated. The presence of conjugation between cells and their buds suggests that the species is homothallic.

Ascopores were observed on 5% malt extract and YM agars after 7–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	w/–		

Co-Q: 7, CBS 5361 (Yamada et al. 1973a).
Mol% G+C: 34.6, 37.6, CBS 5361 (T_m: Nakase and Komagata 1970b; BD: Kurtzman, unpublished).

Origin of the strains studied: Slime flux of cottonwood (*Populus trichocarpa*), California (2).

Type strain: CBS 5361 (NRRL Y-6781).

42.88. *Pichia triangularis* M.Th. Smith & Batenburg-van der Vegte (1986a)

Anamorph: *Candida polymorpha* Ohara & Nonomura ex M.Th. Smith & Batenburg-van der Vegte

Synonyms:
Candida polymorpha Ohara & Nonomura (1954c) nom. nud.
Candida polymorpha Ohara & Nonomura ex M.Th. Smith & Batenburg-van der Vegte (1986a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to ellipsoidal to elongate, (2.1–4.3)×(3.1–9.5) µm, and often with irregular shapes, which most commonly are somewhat triangular or obpyriform. The cells occur singly, in pairs and in small clusters. Growth is butyrous, white to tannish-white, and dull-glistening.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only poorly developed pseudohyphae. True hyphae are not formed. Aerobic growth is butyrous, white to tannish-white, dull, and with a finely striated surface that becomes convoluted. Colonies are raised but with a generally flat surface. A faintly acidic odor is produced.

Formation of ascospores: Asci are unconjugated and form one to four hat-shaped ascospores. Asci deliquesce at maturity. It is unknown whether the species is homothallic or heterothallic.

Ascospore formation was observed on 5% malt extract agar and Difco yeast morphology agar after 5–7 days at 25°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	w
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	w
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	+/w	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 9, *Candida polymorpha* (Yamada and Kondo 1972a).

Mol% G+C: 32.4, 36.6, CBS 4094 (T_m: Nakase and Komagata 1971f, Meyer and Phaff 1972).

Origin of the strain studied: The isolate of Ohara and Nonomura (1954c), from tamari soya, Japan.

Type strain: CBS 4094 (NRRL Y-5714).

Comments: Results from the present study are in agreement with the description of Smith and Batenburg-van der Vegte (1986a) except that salicin was not assimilated. The unusually shaped vegetative cells of *P. triangularis* are somewhat similar to those of *Candida diddensiae*.

42.89. *Pichia veronae* K. Kodama (Kodama & Kyono 1974b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.5–6.5)×(4.9–8.0) µm, and occur singly or in pairs. Growth is cream colored, faintly glistening and butyrous.

Growth on the surface of assimilation media: Thin climbing pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows well-branched pseudohyphae with abundant blastoconidia. True hyphae are not formed. Aerobic growth is white, smooth, faintly glistening and butyrous. Margins may be entire or finely lobed. A faintly acidic odor is produced.

Formation of ascospores: Asci are unconjugated and produce two to four hat-shaped ascospores (Fig. 152). The spores are liberated soon after formation. This species is heterothallic.

Ascospores were observed on 5% malt extract and YM agars after 3–5 days at 25°C.

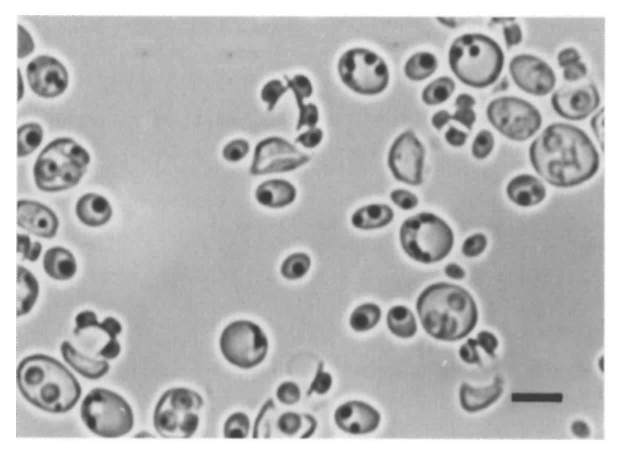


Fig. 152. *P. veronae*, CBS 6591. Ascosporogenous culture after 3 days, 25°C, on 5% malt extract agar. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	w	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	w
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, CBS 6591 (Billon-Grand 1985).
Mol% G+C: 46.6, 49.5, CBS 6591 (T_m: Kodama and Kyono 1974b; BD: Kurtzman 1987a).

Origin of the strain studied: Exudate of the horse chestnut *Aesculus turbinata*, Japan (1).

Complementary mating types: NRRL Y-7818-1 (CBS 6895) and NRRL Y-7818-10 (CBS 6896), derived as single-spore isolates from the type strain (Kurtzman, unpublished).

Type strain: CBS 6591 (LKB-325, NRRL Y-7818).

Comments: Results obtained in the present study correspond well with the original description. Exceptions concern raffinose and L-arabinose on which Kodama (Kodama and Kyono 1974b) found no growth, but which were latently utilized in the present comparison. No mating response has been observed between *P. veronae* and the phenotypically similar species *P. rhodanensis, P. wickerhamii, P. amylophila* and *P. mississippiensis.*

42.90. *Pichia wickerhamii* (van der Walt) Kreger-van Rij (1964b)

Synonym:
Endomycopsis wickerhamii van der Walt (1959b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to elongate, (1.7–5.0)×(2.5–12.0) µm, and occur singly, in pairs and in chains. Growth is tannish-yellow, somewhat wrinkled, and dull to faintly glistening.

Growth on the surface of assimilation media: Pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant well-branched pseudohyphae bearing blastoconidia that may be single or in small verticillate clusters. The presence of true hyphae is questionable. All hyphae examined seemed to have slight constrictions at the septa rather than parallel walls as would be expected of true hyphae. Aerobic growth is light tannish-white, usually dull, wrinkled and somewhat mycelial. Margins are generally entire. An ester-like odor is present.

Formation of ascospores: Asci are unconjugated when formed by diploid strains of this heterothallic species, or conjugated if produced by the pairing of complementary mating types. Two to four hat-shaped ascospores form in each ascus and they are readily liberated.

Ascospores were observed on 5% malt extract agar after 3–7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+/w	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	v
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+/w		

Co-Q: 7, CBS 4107 (Yamada et al. 1973a).
Mol% G+C: 43.2, 45.6, 46.5, CBS 4107 (T_m: Nakase and Komagata 1970b; BD: Fuson et al. 1980, Kurtzman et al. 1980a).

Origin of the strains studied: Frass of snout moth (Pyralidae) larvae in *Encephalartos* sp. (3).

Complementary mating types: NRRL Y-2435-9 (CBS 7100) and NRRL Y-2435-10 (CBS 7101), derived as single-spore isolates from the type strain.

Type strain: CBS 4107 (NRRL Y-2435).

Comments: In comparisons of phenotypically similar

species, *P. wickerhamii* failed to show any mating response with *P. amylophila, P. mississippiensis, P. rhodanensis* or *P. veronae*. Furthermore, *P. wickerhamii* showed only minimal nDNA relatedness with these other species (Kurtzman et al. 1980a).

42.91. *Pichia xylosa* Phaff, M.W. Miller & Shifrine (1956)

Synonym:

Zymopichia xylosa (Phaff, M.W. Miller & Shifrine) Novák & Zsolt (1961)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (1.5–5.0)×(3.0–9.7) µm, and occur singly and in pairs. Growth is yellowish-white, smooth to wrinkled, and dull-glistening.

Growth on the surface of assimilation media: Thin pellicles are formed by some strains.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, branched pseudohyphae with blastoconidia in a verticillate arrangement. True hyphae are not formed. Aerobic growth is tannish-white, smooth to wrinkled, dull-glistening and butyrous. Margins are usually lobed.

Formation of ascospores: Conjugation of a parent cell and a bud usually precedes ascus formation. Asci contain one to four hat-shaped ascospores and these may be quickly or slowly liberated. Single-spore isolates from four-spored asci are ascosporogenous, indicating the species to be homothallic.

Ascospores were observed on 5% malt extract agar after 3–7 days at 25°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	+/w
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+/w	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7, IFO 950 (Yamada et al. 1973a).

Mol% G + C: 39.9–40.4, CBS 2286, 7206, 7207 (BD: Kurtzman 1987a, unpublished).

Origin of the strains studied: CBS 2286 was isolated by Phaff et al. (1956) from a fruit fly (*Drosophila miranda*) in California. NRRL Y-5987, from Boidin, may be a subculture of the type strain. NRRL YB-3884 (CBS 7206) and NRRL YB-3887 (CBS 7207) were isolated by Wickerham from, respectively, the Tuolumne River, Yosemite National Park, California, and a river near Lake Tahoe, California.

Type strain: CBS 2286 (NRRL Y-12939), from *Drosophila miranda.*

Comments: On standard tests, *P. xylosa* is phenotypically indistinguishable from *P. toletana,* which led Kreger-van Rij (1970c) and subsequent workers to consider the taxa to be conspecific. In a comparison of nDNA relatedness between species of *Pichia,* Kurtzman (1992b) found 30% complementarity between *P. xylosa* and *P. toletana,* thus demonstrating the two taxa to be separate but closely related species. The species can be separated from extent of nDNA relatedness, nucleotide divergence in the 26S rRNA gene, or from differences in electrophoretic patterns of cellular proteins (Kurtzman 1992b).

Comments on the genus

Since Hansen established the genus *Pichia* in 1904, the definition of this taxon has changed considerably to accommodate the ever increasing number of new species assigned to it. A historical account of the genus, named in honor of Professor P. Pichi, may be found in the treatments of Lodder and Kreger-van Rij (1952) and Kreger-van Rij (1970c). Many aspects of the earlier taxonomic evolution of *Pichia,* such as ploidy, the presence or absence of a mating system, the type of conjugation, if any, preceding ascus formation, presence of pellicles and the occurrence of gaseous fermentation, were discussed in detail by Kreger-van Rij (1970c).

Electron microscopic and biochemical comparisons have been used to redefine *Pichia* since preparation of the last two major monographs of this genus (Kreger-van Rij 1970c, Kurtzman 1984c,d). Kurtzman and Smiley (1974, 1979) and Kurtzman et al. (1975, 1980b) observed by scanning electron microscopy that, with the exception of *P. carsonii* and *P. etchellsii*, the "round-spored" species of *Pichia* had roughened surfaces. This prompted the reassignment of those species having Co-Q 7 to *Issatchenkia* (Kurtzman et al. 1980b), and those species with Co-Q 9 to *Debaryomyces* (Price and Phaff 1979). *P. carsonii* and *P. etchellsii,* each with Co-Q 9, remained in *Pichia* because their ascospores were glabrous, and

P. fluxuum (Co-Q 7) was retained in *Pichia* because its roughened ascospores, unlike species of *Issatchenkia,* have a subequatorial ring. Nonetheless, use of ascospore morphology as a criterion for the definition of taxa has been uncertain since the demonstration by Wickerham and Burton (1954b) that *P. ohmeri* forms ascospores that are either hat-shaped or spheroidal, depending on which mating types are paired.

As proposed by Stelling-Dekker in 1931, *Hansenula* and *Pichia* were separated from each other primarily on the basis of ability to assimilate nitrate as a sole source of nitrogen. This distinction between the genera was further emphasized by Wickerham (1951). The wisdom of separating the genera on only this one point of difference was discussed (Mrak et al. 1942b, Lodder and Kreger-van Rij 1952, Wickerham 1970a), but because nitrate-variable strains had not been detected among species in either genus, both *Hansenula* and *Pichia* were maintained. In comparisons of nDNA complementarity between phenotypically similar species of *Pichia* and *Hansenula,* Kurtzman (1984a) found 75% relatedness between *P. lindneri* and *H. minuta,* thus demonstrating nitrate assimilation to be a species-variable character. This finding prompted the transfer of *Hansenula* species with hat-shaped ascospores to *Pichia,* the genus of taxonomic priority. The preceding observation concerning nitrate assimilation is not unique and differences in ability to assimilate nitrate also have been detected between the following pairs that show high nDNA relatedness: *Williopsis saturnus* var. *mrakii* (*H. mrakii*) and *W. saturnus* var. *sargentensis* (*P. sargentensis*) (68%, Kurtzman 1991a), strains of *P. canadensis* (95%, Comments on the species, this chapter), and *Sterigmatomyces halophilus* and its synonym *S. indicus* (99%, Kurtzman 1990a).

In 1977, von Arx et al. reintroduced the genus *Williopsis* for saturn-spored species that had been assigned to *Hansenula* (Wickerham 1970a). It was anticipated that the saturn-spored species of *Pichia* would be placed in *Williopsis* as well (Kurtzman 1984a), but comparisons of rRNA sequence divergence demonstrated that this group of species was phylogenetically divergent from *Williopsis,* which prompted their assignment to the newly described genus *Saturnispora* (Liu and Kurtzman 1991).

Billon-Grand (1989) pointed out that species of *Pichia* may be divided into three groups based on the type of coenzyme Q produced (Q-7, Q-8, Q-9), and in view of this, transferred all Q-9 producing species that form hat-shaped ascospores to the newly described genus *Yamadazyma. P. carsonii* and *P. etchellsii,* which also produce Co-Q 9, were left in *Pichia* because they form spheroidal ascospores. Yamada et al. (1992b,d) showed from rRNA sequence comparisons that *P. carsonii* and *P. etchellsii* are closely related to species of *Debaryomyces* and proposed their reassignment to this genus. Kurtzman and Robnett (manuscript in preparation) also compared the rRNA sequences of Co-Q 9-forming yeasts and found species of *Debaryomyces* and some species of *Yamadazyma* to be quite closely related. Because of the uncertain circumscription of these two genera and their unresolved relationships with other genera that produce Co-Q 9, the genus *Yamadazyma* has not been accepted in the present treatment.

Yamada et al. (1994c) compared partial rRNA sequences of nitrate-assimilating species of *Pichia* (species formerly assigned to *Hansenula*), and argued that nitrate assimilation provided an initial grouping that served as a starting point for further dissection by molecular methods. The study revealed seven groupings, three of which were described as the genera *Ogataea, Kuraishia* and *Nakazawaea.* Following this, Yamada et al. (1995a,b) described *Komagataella* for *P. pastoris* and *Kodamaea* for *P. ohmeri*. These genera are not accepted in the present treatment because rRNA comparisons that included all species presently assigned to *Pichia* demonstrated the genus to be comprised of six major clades distributed across the ascomycetous yeast domain, and two of the clades contain closely related nitrate-assimilating and non-assimilating species (Kurtzman and Robnett, manuscript in preparation). Furthermore, there is some overlap with other extant genera that requires resolution.

Although each of the 91 species accepted in the present treatment appears genetically isolated from one another as predicted from genetic crosses, nDNA reassociation, and/or rRNA sequence analyses, it is clear from the foregoing discussions that the genus *Pichia* is polyphyletic, and therefore artificial. Placement of *Pichia* species into phylogenetically defined genera must await molecular comparisons in which all known ascomycetous yeasts are included. The promise of molecular comparisons to provide a phylogenetic framework to yeast systematics has yet to be realized because sequencing studies are laborious and most comparisons to date have focused on phenotypically defined genera, thus inadvertently overlooking other phylogenetically related taxa. For example, the initial characterization of *Debaryomyces* resulted in the inclusion of *Schwanniomyces* (Kurtzman and Robnett 1991). This was followed by the addition of *P. carsonii* and *P. etchellsii* to the genus (Yamada et al. 1992b,d), and *Wingea* was then recognized as a synonym (Kurtzman and Robnett 1994b). In continuation, the relationships of *Pichia* species having hat-shaped ascospores and Co-Q 9 will need to be examined relative to *Debaryomyces.* Further problems that will impact presently accepted genera include the close relationships shown by rRNA/rDNA comparisons for *P. membranifaciens, Issatchenkia orientalis* and *Saturnispora dispora* (Wilmotte et al. 1993, Kurtzman and Robnett 1994a).

Several other methodologies have been used to examine relationships among species assigned to *Pichia.* The application of numerical analysis, proton magnetic resonance, and serology of cell wall mannans has been discussed previously (Kurtzman and Phaff 1987). The use of cellular long-chain fatty acid profiles for recognizing species has been examined in detail by Muller et al. (1987)

and Viljoen et al. (1986a,b, 1988) who reported that it was possible to differentiate between 43 strains representing 33 *Pichia* species. Groupings of species reported in this work do not correspond well with those derived either from rRNA/rDNA comparisons or from coenzyme Q determinations. However, the broader relationships shown by fatty acid profiles for representatives of the entire yeast clade (Botha and Kock 1993) are reasonably congruent with relationships proposed from rRNA/rDNA analyses (Kurtzman and Robnett 1994a, 1995, unpublished). Shen and Lachance (1993) examined the phylogeny of cactophilic *Pichia* species from analysis of rDNA restriction maps. Cactus yeasts that form Co-Q 7 were shown to be closely related to one another and to *P. membranifaciens,* a species common to many habitats.

Species not accepted in the genus

Species for which no strains were available:
Hansenula ellipsoidospora Luteraan (1954)
Pichia moniliformis Nishimura (1910)
Pichia ovaria Nishimura (1910)
Pichia californica (Seifert) Guilliermond (1912)
Pichia megalospora Kuraishi (1958)
Pichia orientalis (Beijerinck) Guilliermond (1912)
Pichia radaisii (Lutz) Guilliermond (1912)
Pichia tamarindorum (Seifert) Guilliermond (1912)
Pichia taurica (Seifert) Guilliermond (1912)
Pichia (Zygopichia) uvarum Verona & Luchetti (1941)
Pichia methanothermo Minami & Yamamura (Minami et al. 1978)

Species for which a validly published description was not located:
Hansenula misumaiensis Sasaki & Yoshida (1958) nom. nud. Strains [CBS 8062 (NRRL Y-17389), CBS 8063 (NRRL Y-17390)] of this taxon did not form ascospores in the present study, and the species has not been transferred to *Pichia.*

Species represented by a mixed culture:
Pichia labacensis Cimerman & Kocková-Kratochvílová (1984). Cultures of this species were reported to be a mixture of *P. membranifaciens* and *Saccharomyces cerevisiae* (Barnett et al. 1990).

43. *Protomyces* Unger

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by budding on a narrow base near the poles of the cell. The cells are ovoidal to elongate, and elongate cells may be asymmetrical. Pseudohyphae and true hyphae are not formed in culture.

The ascosporic state is confined to the tissue of infected plants. Heavy walled spheroidal structures, variously termed ascogenous cells, synasci, and 'chlamydospores' appear to be the site of meiosis. Following nuclear division, which may occur after a resting period, 50–200 ellipsoidal ascospores are formed. Ascospore release may be directly through a slit in the ascogenous cell or an ascus-like vesicle containing the ascospores may emerge through a slit in the ascogenous cell. Ascospores form a yeastlike budding stage. Infection of the host seems only to occur by hyphae which result following conjugation of compatible yeast cells.

Sugars are not fermented. Nitrate is assimilated by most species. Diazonium blue B reaction is negative. Carotenoid pigments are produced. Formation of extracellular starch-like compounds is strong, weak or variable for some species.

Type species

Protomyces macrosporus Unger

Species examined

See Comments on the genus for additional described species.

1. *Protomyces gravidus* Davis (1907)
2. *Protomyces inouyei* Hennings (1902)
3. *Protomyces inundatus* Dangeard (1906)
4. *Protomyces lactucaedebilis* Sawada (1922)
5. *Protomyces macrosporus* Unger (1833)
6. *Protomyces pachydermus* von Thümen (1874)

Key to species

See Table 36.

1. a D-Ribose assimilated .... *P. lactucaedebilis*: p. 355
   b D-Ribose not assimilated → 2
2(1). a L-Arabinose assimilated .... *P. inouyei*: p. 354
   b L-Arabinose not assimilated → 3
3(2). a D-Glucitol assimilated → 4
   b D-Glucitol not assimilated .... *P. macrosporus*: p. 355
4(3). a Salicin assimilated .... *P. gravidus*: p. 354
   b Salicin not assimilated → 5
5(4). a Ascogenous cells germinate without a rest period .... *P. inundatus*: p. 354
   b Ascogenous cells require a rest period before germination .... *P. pachydermus*: p. 356

Table 36
Key characters of species examined in the genus *Protomyces*

Species	Assimilation				Rest period for germination[a]
	D-Ribose	L-Arabinose	D-Glucitol	Salicin	
Protomyces gravidus	–	–	+	+	?
P. inouyei	–	+	+	w	?
P. inundatus	–	–	+	–	no
P. lactucaedebilis	+	+	+	–	?
P. macrosporus	–	–	–	–	?
P. pachydermus	–	–	+	–	yes

[a] Ascogenous cells require/do not require a rest period before ascospore formation.

Systematic discussion of the species

43.1. *Protomyces gravidus* Davis (1907)

Growth on 5% malt extract agar: After 3 days at 20°C, the cells are ellipsoidal to elongate, (2.5–4.0)×(2.5–10.0) μm, and may be tapered or curved. The cells occur singly, in pairs, and in small clusters. Growth is butyrous and dark salmon in color.

Growth on the surface of assimilation media: Incomplete pellicles may form on some media.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is butyrous and glistening with a tannish-orange color. Colony margins are finely and irregularly lobed. A faint acidic odor is present.

Fermentation: absent.

Assimilation (25°C, 28 days):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	s	D-Gluconate	–
Soluble starch	–	DL-Lactate	s
D-Xylose	s	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	s
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
5-Keto-D-gluconate	–	0.01% Cycloheximide	–
Saccharate	–	Growth at 25°C	+
10% NaCl/5% glucose	–	Growth at 37°C	–
Starch formation	w		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: NRRL Y-17093, from giant ragweed (*Ambrosia trifida* L.) Arkansas, U.S.A.
Representative strain: NRRL Y-17093.

43.2. *Protomyces inouyei* Hennings (1902)

Growth on 5% malt extract agar: After 3 days at 20°C, the cells are ellipsoidal to elongate, (2.0–4.0)×(2.5–10.0) μm, and may be tapered, curved or of unusual shapes. The cells occur singly, in pairs, and in small clusters. Growth is butyrous and salmon colored.

Growth on the surface of assimilation media: Incomplete pellicles may form on some media.

Dalmau plate culture on morphology agar: After 7 days at 20°C, growth under the coverglass shows neither true hyphae nor pseudohyphae. Aerobic growth is butyrous, glistening and tannish-orange in color. Colony margins are irregularly lobed. A faint acidic odor is present.

Fermentation: absent.

Assimilation (20°C, 28 days):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	s	Glycerol	s
Maltose	s	Erythritol	–
Cellobiose	s	Ribitol	s
Trehalose	s	Galactitol	w/–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	s	α-Methyl-D-glucoside	s
Melezitose	s	Salicin	w
Inulin	s	D-Gluconate	–
Soluble starch	s	DL-Lactate	s
D-Xylose	s	Succinate	s
L-Arabinose	s	Citrate	w/–
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	w/–
L-Rhamnose	–	Nitrate	v
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	w
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	0.01% Cycloheximide	n
10% NaCl/5% glucose	–	Growth at 37°C	–

Co-Q: 10, IFO 6627 (Yamada et al. 1983).
Mol% G+C: 52.0, IFO 6627 (T_m: Nakase and Komagata 1971g).

Origin of the strains studied: NRRL Y-6347, from L.R.G. Valadon, England; NRRL YB-4354, from K. Tubaki, parasitic on *Crepis japonica,* Japan.
Representative strain: NRRL YB-4354.

43.3. *Protomyces inundatus* Dangeard (1906)

Growth on 5% malt extract agar: After 3 days at 20°C, the cells are ellipsoidal to elongate, (2.0–4.7)×(3.7–9.0) μm, and may be tapered, curved or of unusual shapes (Fig. 153). The cells occur singly, in pairs, and in small clusters. Growth is butyrous and light salmon colored.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 20°C, growth under the coverglass shows neither true hyphae nor pseudohyphae. Aerobic growth is butyrous, glistening and tannish-orange in color. Colony

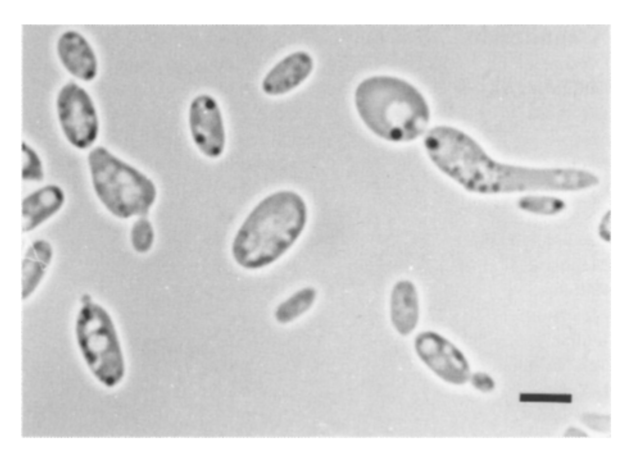


Fig. 153. *P. inundatus*, NRRL Y-6802. After 3 days on YM agar at 15°C. Bar = 5 μm.

margins are irregularly lobed. A faint acidic odor is present.

Fermentation: absent.

Assimilation (20°C, 28 days):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	–
Inulin	+	D-Gluconate	–
Soluble starch	+	DL-Lactate	w/–
D-Xylose	+	Succinate	+/w
L-Arabinose	–	Citrate	+/w
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	w/–
5-Keto-D-gluconate	–	Gelatin liquefaction	v
Saccharate	–	0.01% Cycloheximide	–
10% NaCl/5% glucose	–	Growth at 25°C	–

Co-Q: Not determined.
Mol% G + C: Not determined.

Origin of the strains studied: NRRL Y-6349, NRRL Y-6802, NRRL Y-6803, from L.R.G. Valadon, England, parasitic on *Apium nodiflorum*.

Complementary mating types: NRRL Y-6802 and NRRL Y-6803.

Representative strain: NRRL Y-6349.

43.4. *Protomyces lactucaedebilis* Sawada (1922)

Growth on 5% malt extract agar: After 3 days at 20°C, the cells are ellipsoidal to elongate, (2.5–5.0) × (3.5–9.0) μm, and often tapered. The cells occur singly, in pairs, and in small clusters. Growth is butyrous and light salmon colored.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 20°C, growth under the coverglass shows neither true hyphae nor pseudohyphae. Aerobic growth is butyrous, glistening and tannish-orange in color. Colony margins are irregularly lobed. A faint acidic odor is present.

Fermentation: absent.

Assimilation (15°C, 50 days):

Glucose	+	*N*-Acetyl-D-glucosamine	s
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	ws
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	s	Ribitol	s
Trehalose	+	Galactitol	s
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	+	D-Gluconate	–
Soluble starch	s	DL-Lactate	s
D-Xylose	s	Succinate	+
L-Arabinose	s	Citrate	+
D-Arabinose	s	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	s
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	w/–
5-Keto-D-gluconate	–	Gelatin liquefaction	w
Saccharate	–	0.01% Cycloheximide	–
10% NaCl/5% glucose	–	Growth at 25°C	+

Co-Q: 10, IFO 6899 (Yamada et al. 1983).
Mol% G + C: 52.0, IFO 6899 (T_m: Nakase and Komagata 1971g).

Origin of the strain studied: NRRL YB-4353, received from K. Tubaki, Japan.

Representative strain: NRRL YB-4353.

43.5. *Protomyces macrosporus* Unger (1833)

Growth on 5% malt extract agar: After 3 days at 20°C, the cells are ellipsoidal to elongate, (2.5–4.0) × (3.0–7.0) μm, and often tapered. The cells occur singly, in pairs, and in small clusters. Growth is butyrous and light salmon colored.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 20°C, growth under the coverglass is devoid of either true hyphae or pseudohyphae. Aerobic growth is

butyrous, glistening and tannish-orange in color. Colony margins are irregularly lobed. A faint acidic odor is present.

Fermentation: absent.

Assimilation (20°C, 28 days):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	+	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	–
5-Keto-D-gluconate	–	0.01% Cycloheximide	–
Saccharate	–	Growth at 20°C	+
10% NaCl/5% glucose	–	Growth at 25°C	n
Starch formation	w		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: NRRL Y-12879, from a leaf gall of hedge parsley [*Torilis japonica* (Houtt) DC.], Arkansas, U.S.A.

Representative strain: NRRL Y-12879.

43.6. *Protomyces pachydermus* von Thümen (1874)

Growth on 5% malt extract agar: After 3 days at 20°C, the cells are ellipsoidal to elongate, (2.5–4.0)×(3.0–8.2) μm, and frequently tapered. The cells occur singly, in pairs, and in small clusters. Growth is butyrous and light salmon colored.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 15°C, growth under the coverglass has neither true hyphae nor pseudohyphae. Aerobic growth is butyrous, glistening and tannish-orange in color. Colony margins are irregularly lobed. A faint acidic odor is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	–
Inulin	+	D-Gluconate	–
Soluble starch	w/–	DL-Lactate	w/–
D-Xylose	v	Succinate	w/–
L-Arabinose	–	Citrate	w
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	v
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Gelatin liquefaction	w
5-Keto-D-gluconate	–	0.01% Cycloheximide	–
Saccharate	–	Growth at 20°C	+
10% NaCl/5% glucose	–	Growth at 25°C	–
Starch formation	+		

Co-Q: 10, IFO 6628 (Yamada et al. 1983).
Mol% G+C: 52.4, IFO 6628 (T_m: Nakase and Komagata 1971g).

Origin of the strains studied: NRRL YB-4355, from K. Tubaki, Japan; NRRL Y-6348, from L.R.G. Valadon, England.

Representative strain: NRRL YB-4355.

Comments on the genus

Species of the genus *Protomyces* are plant pathogens causing tissue distortion similar to that produced by *Taphrina* spp. Members of both genera show budding, yeastlike growth on laboratory media but do not develop hyphae or sexual states unless infecting their host plants (Tubaki 1957, Valadon et al. 1962, von Arx et al. 1982). Recently, species of *Protomyces* became of interest as weed biocontrol organisms (Valverde and Templeton 1984, Cartwright and Templeton 1988).

Species of *Protomyces* generally have a reddish, salmon-like color in culture whereas *Taphrina* species often show lighter shades of red, lavender or yellow. The colors arise from the presence of carotenoids. Van Eijk and Roeymans (1982) compared the carotenoid content of representative species of *Protomyces* and *Taphrina*, and concluded that the two genera cannot be reliably separated from quantitative differences in carotenoid content.

The placement of *Protomyces* among the fungi has been problematical, and Reddy and Kramer (1975) have reviewed earlier ideas on relationships noting that more recent workers have suggested that *Protomyces* is a primitive ascomycete. Molecular comparisons have brought a more definitive perspective to the phylogenetic

placement of *Protomyces*. Walker (1985a) analyzed 5S rRNA sequences and placed *Protomyces* near *Schizosaccharomyces* rather than with either the budding yeasts or the euascomycetes. Kurtzman (1993c) confirmed this placement from analysis of partial 18S and 26S rRNA sequences and noted that *Taphrina* and *Protomyces* are closely related. Nishida and Sugiyama (1994) proposed from comparisons of 18S rDNA gene sequences that *Protomyces, Taphrina, Saitoella, Schizosaccharomyces* and *Pneumocystis* represent a major lineage within the Ascomycota that is basal to the budding yeasts and euascomycetes, and suggested the name Archiascomycetes for this group.

Reddy and Kramer (1975) placed the genera *Burenia, Protomyces, Protomycopsis, Taphridium* and *Volkaria* in the Protomycetaceae, the only family of the Protomycetales, but questioned whether the genus *Mixia* was a member of this family. Since then, Nishida et al. (1995) showed from 18S rDNA comparisons that *Mixia osmundae* is actually a basidiomycete.

Species of *Protomyces* are primarily defined from morphology and host range. Reddy and Kramer (1975) accepted 10 species and listed 61 other described species as doubtful or unavailable for study. These authors also transferred *P. inundatus* to the genus *Burenia.*

The present treatment of *Protomyces* is not intended to be definitive, nor is it certain that the six species described are biologically distinct from one another. The six taxa listed are available from culture collections and serve to illustrate the cultural characteristics of some better known members of the genus. Genetic and molecular comparisons will be needed to understand species boundaries, and for this reason no synonyms are given with the species listed.

44. *Saccharomyces* Meyen ex Reess

Ann Vaughan-Martini and Alessandro Martini

Diagnosis of the genus (Yarrow 1984a)

Vegetative reproduction is by multilateral budding. Cells are globose, ellipsoidal or cylindroidal. Pseudohyphae may be formed but not septate hyphae.

The vegetative phase is predominantly diploid (or of higher ploidy), conjugation occurs on or soon after germination of the ascospores; diploid ascospores may be formed. Ascospores are globose to short ellipsoidal, with a smooth wall and usually one to four per ascus. Asci are persistent.

Vigorous fermentation of sugars. Starch-like compounds are not produced. Absence of growth with nitrate as a sole source of nitrogen. Diazonium blue B reaction is negative.

Type species

Saccharomyces cerevisiae Meyen ex Hansen

Species accepted

1. *Saccharomyces barnettii* Vaughan-Martini (1995)
2. *Saccharomyces bayanus* Saccardo (1895)
3. *Saccharomyces castellii* Capriotti (1966)
4. *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (1883)
5. *Saccharomyces dairenensis* Naganishi (1917)
6. *Saccharomyces exiguus* Reess ex E.C. Hansen (1888)
7. *Saccharomyces kluyveri* Phaff, M.W. Miller & Shifrine (1956)
8. *Saccharomyces paradoxus* Bachinskaya (1914)
9. *Saccharomyces pastorianus* E.C. Hansen (1904)
10. *Saccharomyces rosinii* Vaughan-Martini, Barcaccia & Pollacci (1996)
11. *Saccharomyces servazzii* Capriotti (1967)
12. *Saccharomyces spencerorum* Vaughan-Martini (1995)
13. *Saccharomyces transvaalensis* van der Walt (1956)
14. *Saccharomyces unisporus* Jörgensen (1909)

Key to species

See Table 37.

1.	a	Maximum growth temperature above 30°C → 3		
	b	Growth absent above 30°C → 2		
2(1).	a	Sucrose, raffinose and trehalose fermented	*S. barnettii*:	p. 359
	b	Sucrose, raffinose and trehalose not fermented	*S. rosinii*:	p. 367
3(1).	a	Ethylamine·HCl assimilated → 4		
	b	Ethylamine·HCl not assimilated → 6		
4(3).	a	Growth in the presence of 1000 ppm cycloheximide	*S. unisporus*:	p. 370
	b	Absence of growth in the presence of 1000 ppm cycloheximide → 5		
5(4).	a	Maltose, raffinose and ethanol assimilated	*S. kluyveri*:	p. 365
	b	Maltose, raffinose and ethanol not assimilated	*S. spencerorum*:	p. 368
6(3).	a	Maltose assimilated → 7		
	b	Maltose not assimilated → 10		
7(6).	a	Growth in vitamin-free medium	*S. bayanus*:	p. 360
	b	Absence of growth in vitamin-free medium → 8		
8(7).	a	D-Mannitol assimilated; maximum growth temperature 37°C or greater	*S. paradoxus*:	p. 366
	b	D-Mannitol not assimilated; maximum growth temperature less than 37°C or variable at 37°C → 9		
9(8).	a	Active transport mechanism for fructose present; maximum growth temperature 34°C or below	*S. pastorianus*:	p. 367
	b	Active transport mechanism for fructose not present; maximum growth temperature variable	*S. cerevisiae*:	p. 361
10(6).	a	Sucrose, raffinose and trehalose fermented	*S. exiguus*:	p. 364
	b	Sucrose, raffinose and trehalose not fermented → 11		
11(10).	a	Growth in the presence of 1000 ppm cycloheximide	*S. servazzii*:	p. 368
	b	Absence of growth in the presence of 1000 ppm cycloheximide → 12		

12(11). a D-Ribose normally assimilated; 8–10 chromosomes 600 → 3000 kilobases .... *S. castellii*: p. 360
b D-Ribose not assimilated, mostly single, highly refringent ascospores on acetate agar; 8 chromosomes 400 → 2200 kilobases .... *S. transvaalensis*: p. 369
c D-Ribose normally not assimilated; 7–9 chromosomes 750 → 3000 kilobases .... *S. dairenensis*: p. 363

Table 37
Key characters of species of the genus *Saccharomyces*

Species	Fermentation[a]			Assimilation[a]									Growth[a]				Frc[b]
	Su	Raf	Tr	Carbon source						N source			Cychx 1000	30°C	37°C	Vit-free	
				Su	Ma	Raf	DRi	Eth	DM	Cad	Ety	Lys					
Saccharomyces barnettii	+	+	s	+	–	+	–	–	–	–	–	–	–	–	–	–	n
S. bayanus	+	+	–	+	+	+	–	+	v	–	–	–	–	+	–	+	+
S. castellii	–	–	–	–	–	–	+/v	–	–	–	–	–	–	+	v	–	n
S. cerevisiae	+	+	–	+	+	+	–	+	–	–	–	–	–	+	v	–	–
S. dairenensis	–	–	–	–	–	–	v	v	–	–	–	–	–	+	v	–	n
S. exiguus	+	s	+	+	–	+	–	s	–	–	–	–	v	+	–	–	n
S. kluyveri	+	+	–	+	+	+	–	+	+	+	+	+	–	+	+	–	n
S. paradoxus	+	+	–	+	+	+	–	+	+	–	–	–	–	+	+	–	–
S. pastorianus	v	+	–	+	+	+	–	+	–	–	–	–	–	+	–	–	+
S. rosinii	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	n
S. servazzii	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	n
S. spencerorum	+	–	+	+	–	–	–	–	–	+	+	+	–	+	+	–	n
S. transvaalensis	–	–	–	–	–	–	–	v	–	v	–	v	–	+	+	–	n
S. unisporus	–	–	–	–	–	–	–	+	–	+	+	+	+	+	v	–	n

[a] Abbreviations: Su, sucrose; Raf, raffinose; Tr, trehalose; Ma, maltose; D-Ri, D-ribose; Eth, ethanol; DM, D-mannitol; Cad, cadaverine·2HCl; Ety, ethylamine·HCl; Lys, L-lysine; Cychx 1000, indicates resistance to 1000 ppm cycloheximide in the medium; Vit-free, growth in vitamin-free medium.

[b] Presence of a fructose transport system; n, not determined.

Systematic discussion of the species

44.1. *Saccharomyces barnettii* Vaughan-Martini (1995)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ovoidal or elongate, (2.2–6.0)×(3.5–10.5) μm, and are usually isolated, or in pairs. After one month at 20°C, a sediment is present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is butyrous and light cream-colored. The surface is smooth, usually flat, occasionally raised or folded and opaque.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are not present.

Formation of ascospores: Vegetative cells are transformed directly into persistent asci containing one or two, rarely four, globose to short ellipsoidal ascospores.

Ascospores were observed on acetate agar usually after 20–40 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	s
Sucrose	+	Trehalose	s
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	–
L-Lysine	–	Growth at 37°C	–
Ethylamine·HCl	–		

Co-Q: Not determined.
Mol% G+C: 33.4, CBS 6946 (T_m: Yarrow 1978).

Origin of the strains studied: DBVPG 6365 (CBS 5648), sauerkraut; DBVPG 6370 (CBS 6946), soft drink.

Type strain: CBS 5648 (DBVPG 6365) isolated from sauerkraut.

Comments: Strains of this species require biotin for growth (Yarrow, personal communication). For additional comments see the general discussion under *S. exiguus.*

44.2. *Saccharomyces bayanus* Saccardo (1895)

Synonyms:

Saccharomyces uvarum Beijerinck (1898b)
Saccharomyces globosus Osterwalder (1924a)
Saccharomyces heterogenicus Osterwalder (1924a)
Saccharomyces intermedius E.C. Hansen var. *valdensis* Osterwalder (1924a)
Saccharomyces tubiformis Osterwalder (1924a)
Saccharomyces inusitatus van der Walt (1965b)
Saccharomyces abuliensis Santa Maria (1978)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ovoidal or elongate, (2.2–6.0)×(3.5–10.5) µm, and are usually isolated, but can be in groups of 3–20 cells (Fig. 154). After one month at 20°C, a sediment is present.

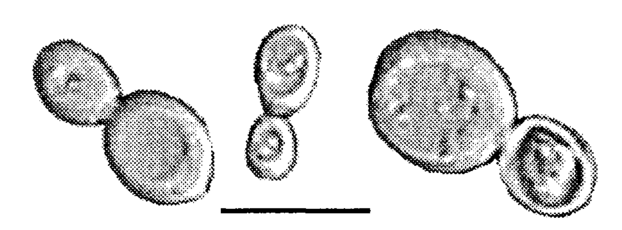


Fig. 154. *S. bayanus*, CBS 380. Vegetative cells after 2 days, on YEPG agar at 25°C. Bar = 10 µm.

Growth on 5% malt agar: After one month at 20°C, the streak culture is butyrous and light cream-colored. The surface is smooth, usually flat, occasionally raised or folded and opaque.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 5 days at 25°C, well-developed pseudohyphae are usually present.

Formation of ascospores: Vegetative cells are transformed directly into persistent asci containing one to four globose to short ellipsoidal ascospores.

Ascospores were observed only on acetate agar. After 14 days at 25°C, sporulation is usually below 10% and ascospores are rarely viable.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	+	Melibiose	v

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	v	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	–
L-Lysine	–	Active fructose transport	+
Ethylamine·HCl	–	Growth at 37°C	–

Co-Q: 6 (Yamada et al. 1976b).

Mol% G+C: 37–40, NRRL Y-409, NRRL Y-969 (T_m: Bicknell and Douglas 1970); 38.8–40.5, AJ 4068, AJ 5274, AJ 5308, AJ 5309, AJ 5351, AJ 5357 (T_m: Nakase and Komagata 1971e); 41.6–42.1, CBS 380, CBS 395, CBS 1546 (BD: Vaughan-Martini and Kurtzman 1985); 37.8–41.5, CBS 380, CBS 395, CBS 431 (T_m: Vaughan-Martini, unpublished data).

Origin of the strains studied: DBVPG 6032 (CBS 378), unknown; DBVPG 6131 (CBS 431), type strain of *S. tubiformis*, fermenting pear juice; DBVPG 6171 (CBS 380), beer; DBVPG 6179 (CBS 395), type strain of *S. uvarum*, berries; DBVPG 6253 (CBS 1546), type strain of *S. inusitatus*, beer; DBVPG 6259 (CBS 1604), perry; DBVPG 6260 (CBS 1505), type strain of *S. intermedius* var. *valdensis*, grape must; DBVPG 6347 (NCYC 374), unknown.

Type strain: CBS 380 (DBVPG 6171, IFO 1127, NRRL Y-12624), isolated from beer, received by CBS from the Central Laboratory of the South Manchuria Railway Company.

44.3. *Saccharomyces castellii* Capriotti (1966)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ovoidal or elongate, (3.0–5.5)×(3.5–7.0) µm, single or in pairs. After one month at 20°C, a sediment is present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is butyrous and light cream-colored. The surface is smooth and glossy and the margin is entire or crenate.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are not formed.

Formation of ascospores: Vegetative cells are transformed directly into persistent asci containing one or two, rarely four, globose ascospores.

Sporulation was observed on acetate agar and YM agar in some strains after 7–9 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	–
L-Lysine	–	Growth at 37°C	v
Ethylamine·HCl	–		

Co-Q: Not determined.

Mol% G+C: 38.8, CBS 4309 (T_m: Yarrow and Nakase 1975); 36.7–39.1, CBS 4309, CBS 4310 (BD: Vaughan-Martini and Kurtzman 1988).

Origin of the strains studied: DBVPG 6298 (CBS 4309), type strain of *S. castellii*, soil; DBVPG 6352 (CBS 3007), DBVPG 6353 (CBS 2913), and DBVPG 6356 (CBS 3006), fermenting cucumber brine; DBVPG 6361 (CBS 4310), soil; DBVPG 6410 (CBS 1579), buttermilk; DBVPG 6751 (CBS 7188), ensiled maize; DBVPG 6758 (CBS 2248), soil; DBVPG 6759 (CBS 4906), caecal contents of a baboon.

Type strain: CBS 4309 (DBVPG 6298, IFO 1992, NRRL Y-12630) isolated by Capriotti from Finnish soil.

Comments: Spectrophotometric DNA reassociation studies (Vaughan-Martini and Kurtzman 1988) revealed that the nucleotide sequences of *S. dairenensis* and *S. castellii* were not homologous, therefore indicating the presence of two separate taxa despite essentially identical physiological responses.

44.4. *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (1883)

Anamorph: *Candida robusta* Diddens & Lodder

Synonyms:

Saccharomyces ellipsoideus Meyen ex E.C. Hansen (1883)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *ellipsoideus* (E.C. Hansen) Dekker (Stelling-Dekker 1931)
Saccharomyces ilicis Grönlund (1892)
Saccharomyces vini-muntz Kayser (1892)
Saccharomyces vordermanii Went & Prinsen Geerligs (1894)
Saccharomyces willianus Saccardo (1895)
Saccharomyces sake Yabe (1897)
Saccharomyces cratericus Lindner (1898a)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *cratericus* (Lindner) Lodder (1932)
Saccharomyces awamori Inui (1901)
Saccharomyces cerevisiae agavica sylvestre Carbajal (1901)
Saccharomyces intermedius E.C. Hansen (1904)
Saccharomyces turbidans E.C. Hansen (1904)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *turbidans* (E.C. Hansen) Dekker (Stelling-Dekker 1931)
Saccharomyces validus E.C. Hansen (1904)
Saccharomyces brasiliensis Lindner (1905b)
Saccharomyces thermantitonum Johnson (1905)
Saccharomyces batatae Saito (1907)
Saccharomyces logos van Laer & Denamur ex Jörgensen (1909)
Saccharomyces multisporus Jörgensen (1909)
Saccharomyces tokyo Nakazawa (1909)
Saccharomyces yedo Nakazawa (1909)
Saccharomyces coreanus Saito (1910)
Saccharomyces shaoshing Takahashi (1911)
Saccharomyces anamensis Will & Heinrich (Will 1913)
Saccharomyces chevalieri Guilliermond (1914)
Saccharomyces lindneri Guilliermond (1914)
Saccharomyces chevalieri Guilliermond var. *lindneri* (Guilliermond) Dekker (Stelling-Dekker 1931)
Saccharomyces mandshuricus Saito (1914)
Saccharomyces mangini Guilliermond (1914)
Saccharomyces marchalianus Kufferath (1920)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *marchalianus* (Kufferath) Dekker (Stelling-Dekker 1931)
Saccharomyces cerasi Schweizer (1921)
Saccharomyces odessa Schnegg & Oehlkers (1922)
Saccharomyces chodati Steiner (1924) [non *Saccharomyces chodati* Schweizer (1921)]
Saccharomyces oviformis Osterwalder (1924a)
Saccharomyces valesiacus Osterwalder (1924a)
Saccharomyces annulatus Negroni (1929)
Saccharomyces festinans Ward & Baker (1929)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *festinans* (Ward & Baker) Dekker (Stelling-Dekker 1931)
Saccharomyces eryobotryae Suminoe & Miwa (1930)
Saccharomyces carlsbergensis E.C. Hansen var. *mandshuricus* (Saito) Dekker (Stelling-Dekker 1931)
Saccharomyces carlsbergensis E.C. Hansen var. *monacensis* (E.C. Hansen) Dekker (Stelling-Dekker 1931)
Saccharomyces carlsbergensis E.C. Hansen var. *polymorphus* Dekker (Stelling-Dekker 1931)
Saccharomyces carlsbergensis E.C. Hansen var. *valdensis* (Osterwalder) Dekker (Stelling-Dekker 1931)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *pulmonalis* (Redaelli) Dekker (Stelling-Dekker 1931)
Saccharomyces elongatus Krumbholz (1932)
Saccharomyces cheresiensis Prostoserdov & Afrikian (1933)
Saccharomyces oviformis Osterwalder var. *cheresiensis* (Prostoserdov & Afrikian) Kudryavtsev (1954)
Saccharomyces formosensis Nakazawa (1933)
Saccharomyces mangini Guilliermond var. *casei* Sacchetti (1933)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *onychophilus* Zach (Wolfram and Zach 1934b)
Saccharomyces intermedius E.C. Hansen var. *turicensis* Osterwalder (1934)

Saccharomyces ellipsoideus E.C. Hansen var. *umbra* Castelli (1935) nom. nud.
Saccharomyces praecisus Nakazawa & Simo (1936)
Saccharomyces robustus Nakazawa & Simo (1936)
Saccharomyces carbajali Ruiz (1938)
Saccharomyces ellipsoideus E.C. Hansen var. *major* Castelli (1938) nom. nud.
Saccharomyces italicus Castelli (1938) nom. nud.
Saccharomyces oviformis Osterwalder var. *bisporus* Castelli (1938) nom. nud.
Saccharomyces mangini Guilliermond var. *miso* Mogi (1942)
Candida robusta Diddens & Lodder (1942)
Mycotorula robusta (Diddens & Lodder) Krasil'nikov (1954c)
Saccharomyces joannae von Szilvinyi & Kaulich (1948)
Saccharomyces fructuum Lodder & Kreger-van Rij (1952)
Saccharomyces hutensis Kufferath ex Dekker (Lodder and Kreger-van Rij 1952)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *fructuum* (Lodder & Kreger-van Rij) Jensen (1967)
Saccharomyces steineri Lodder & Kreger-van Rij (1952)
Saccharomyces capensis van der Walt & Tscheuschner (1956d)
Saccharomyces italicus Castelli var. *melibiosi* van Uden & Assis-Lopes (1957)
Saccharomyces oleaceus Santa María (1958a)
Saccharomyces oleaginosus Santa María (1958a)
Saccharomyces aceti Santa María (1959a)
Saccharomyces oxidans Santa María (1959a)
Saccharomyces carlsbergensis Hansen var. *alcoholophila* Shehata (1960)
Saccharomyces prostoserdovii Kudryavtsev (1960)
Saccharomyces uvarum Beijerinck var. *carlsbergensis* Kudryavtsev (1960)
Saccharomyces uvarum Beijerinck var. *melibiosus* Kudryavtsev (1960)
Saccharomyces vini Meyen ex Kudryavtsev (1960)
Saccharomyces vini Meyen ex Kudryavtsev var. *cartilaginosus* Kudryavtsev (1960)
Saccharomyces cartilaginosus Lindner var. *italiens* Sacchetti (1932a)
Saccharomyces vini Meyen ex Kudryavtsev var. *cerevisiae* Kudryavtsev (1960)
Saccharomyces hienipiensis Santa María (1962)
Saccharomyces norbensis Santa María (1963a)
Saccharomyces onubensis Santa María (1964)
Saccharomyces diastaticus Andrews & Gilliland ex van der Walt (1965a)
Saccharomyces hispanica Santa María (1968)
Saccharomyces beticus Marcilla ex Santa María (1970)
Saccharomyces cartilaginosus Lindner var. *cartilaginosus* Lodder (1970)
Saccharomyces cordubensis Santa María (1970)
Saccharomyces gaditensis Santa María (1970)
Saccharomyces peka Takeda (Lodder 1970)
Saccharomyces acidosaccharophillii Kawano, Kojima, Ohosawa & Morinaga (1976)
Saccharomyces abuliensis Santa María (1978)
Saccharomyces hispalensis Santa María (1978)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *pelliculosa* S. Goto (Barnett et al. 1983)
Saccharomyces boulardii Surawicz, Elmer, Speelman, McFarland, Chinn & van Beele (1989) nom. nud.

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ovoidal or elongate, (3.0–8.0)×(5.0–10.0) µm, and are usually isolated or in small groups (Fig. 155). After one month at 20°C, a sediment is present.

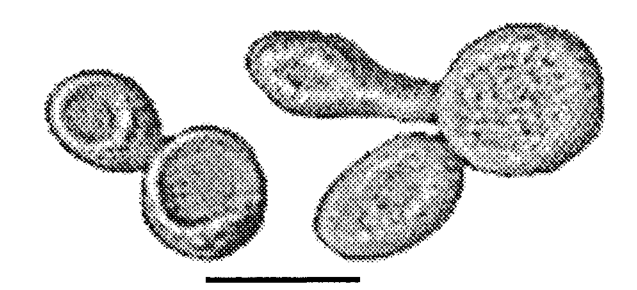


Fig. 155. *S. cerevisiae*, CBS 1171. Vegetative cells after 2 days, on YEPG agar, 25°C. Bar = 10 µm.

Growth on 5% malt agar: After one month at 20°C, growth is butyrous and light cream-colored. The surface is smooth, usually flat, occasionally raised or folded and opaque.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are either not formed or are rudimentary.

Formation of ascospores: Vegetative cells are transformed directly into persistent asci containing one to four globose to short ellipsoidal ascospores (Fig. 156).

Ascospore formation, observed almost exclusively on acetate agar, was usually below 10% except in highly fertile homothallic strains where sporulation ranged from 40–95% in 6–10 days at 20°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	v	Melibiose	v

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	v	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	–
L-Lysine	–	Active fructose transport	–
Ethylamine·HCl	–	Growth at 37°C	v

Co-Q: 6 (Yamada et al. 1976b).
Mol% G+C: 39–41, NRRL Y-123, NRRL Y-379, NRRL Y-1345, NRRL Y-1356, NRRL Y-1373, (T_m: Bicknell and Douglas 1970); 38.5–40.7, 30 strains (T_m: Nakase and Komagata 1971e); 39.0–40.2, CBS 1395, CBS 2247, CBS 3081, CBS 3093, CBS 4903, CBS 5155, CBS 5835 (T_m: Yarrow and Nakase 1975); 39.9, UCD-FST 74-83 (BD: Price et al. 1978); 40.1–41.1, CBS 400, CBS 459, CBS 1171, CBS 1636, CBS 4054, NRRL Y-11846 (BD: Vaughan-Martini and Kurtzman 1985).

Origin of the strains studied: DBVPG 1357, DBVPG 1360, DBVPG 1361, DBVPG 1772, DBVPG 1776, DBVPG 1789 and DBVPG 1793, vineyard soil; DBVPG 1948 and DBVPG 1950, cavern soil; DBVPG 6029 (CBS 1907), type strain of *C. robusta*, DBVPG 6037 (CBS 2980), *Drosophila*; DBVPG 6039 (CBS 1395), type strain of *S. ellipsoideus*, DBVPG 6042 (CBS 403), type strain of *S. lindneri*, ginger beer; DBVPG 6043 (CBS 382), type strain of *S. logos*, brewery; DBVPG 6046 (CBS 422), type strain of *S. odessa*, beer; DBVPG 6048 (CBS 423), type strain of *S. steineri*, wine; DBVPG 6173 (CBS 1171), beer; DBVPG 6220 (CBS 6503), brewery killer; DBVPG 6248 (CBS 2354) (NCYC 74) used for assay of pyridoxine and pantothenic acid; DBVPG 6249 (CBS 2444), distillery; DBVPG 6250 (CBS 4734), sugar cane; DBVPG 6251 (CBS 1250) type strain of *S. cheresiensis*, sherry; DBVPG 6254 (CBS 429) type strain of *S. oviformis*, grape must.

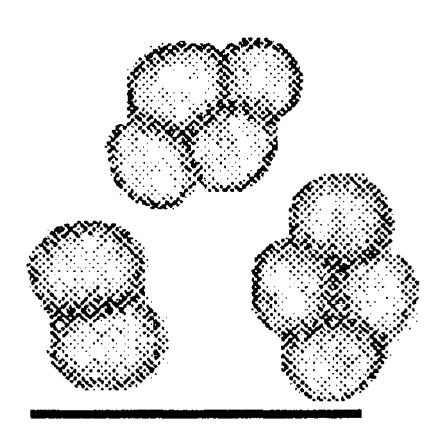


Fig. 156. *S. cerevisiae*, CBS 403. Unconjugated asci with ascospores after 7 days on McClary's acetate agar, 25°C. Bar = 10 μm.

Complementary mating types: CBS 1782.1 and CBS 1782.2 or CBS 2354.1 and CBS 2354.2.

Type strain: CBS 1171 (ATCC 18824, DBVPG 6173, NCYC 505, NRRL Y-12632), isolated by van Wijk from the top yeast used in the Oranjeboom Brewery at Rotterdam, and designated as the type strain by Lodder and Kreger-van Rij (1952).

Comments: A large number of ecological and technological investigations have set forth the hypothesis that *S. cerevisiae* is the principal species responsible for the alcoholic fermentation of fruit juices. The traditional epithets such as *S. ellipsoideus, S. oviformis, S. cheresiensis* or *S. chevalieri*, retained over the years as the main species in this process, have been shown to be strains of *S. cerevisiae* with particular technological capabilities. In addition, other members of the *sensu stricto* complex: *S. bayanus, S. paradoxus,* and *S. pastorianus* (*sensu* Vaughan-Martini and Martini 1987a, Vaughan-Martini 1989), while at times found in these man-created environments, do not appear to be the most capable starters for the process.

Since the discovery that some strains of *S. cerevisiae* are capable of producing a "killer" toxin lethal to other members of the same species (Bevan and Makover 1963), many investigations have been carried out on the mode of action and the chemical and physical properties of the toxin as well as the biology of the "killer" plasmid. It has been asserted that this phenomenon can be technologically useful, and numerous studies have been tested in this regard. On the other hand, it is not possible to apply this character in yeast classification since studies have shown that this is a strain- rather than species-related character (Vaughan-Martini and Rosini 1989).

The *nomen nudum S. boulardii*, cited in recent years as an anti-diarrhea agent (Surawicz et al. 1989), is homologous with *S. cerevisiae* for 95% of its genome (Vaughan-Martini, unpublished data).

44.5. *Saccharomyces dairenensis*[1] Naganishi (1917)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ovoidal or elongate, (3.0–5.5)×(3.5–7.0) μm, single or in pairs. After one month at 20°C, a sediment is present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is butyrous and light cream-colored. The surface is smooth and glossy and the margin is entire or crenate.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: No pseudohyphae are formed.

Formation of ascospores: Vegetative cells are transformed directly into persistent asci containing one or two, rarely four, globose ascospores.

Sporulation was observed on acetate agar and YM agar in some strains after 7–9 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Melibiose	–

[1] The original spelling of the species epithet *dairensis* has been treated as an orthographic error.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	s
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	–
L-Lysine	–	Growth at 37°C	v
Ethylamine·HCl	–		

Co-Q: Not determined.

Mol% G+C: 37, NRRL Y-1353 (T_m: Bicknell and Douglas 1970); 35.9–38.5, 8 strains (T_m: von Arx 1977b, 1978); 36.7, CBS 421 (BD: Vaughan-Martini and Kurtzman 1988).

Origin of the strains studied: DBVPG 6357 (CBS 6904), soil; DBVPG 6359 (CBS 6463), fermenting grapes; DBVPG 6366 (CBS 421), dry fruit of a persimmon; DBVPG 6752 (CBS 6334), fermenting mushroom.

Type strain: CBS 421 (ATCC 10597, DBVPG 6366, IFO 1168, NRRL Y-1353) isolated by Naganishi.

Comments: DNA/DNA reassociation (Vaughan-Martini and Barcaccia 1996) has shown that many strains previously classified as *S. dairenensis* are homologous to the type strain of the species *S. castellii* with which they must now be considered synonymous. The same study demonstrated that none of the strains examined shared significant DNA base sequences with the type strain of *S. dairenensis*. Nevertheless, due to the limited response of strains of this group to the tests of conventional taxonomy, it is not possible at the present time to establish a clear distinction between all but one of these separate species. As a consequence, until satisfactory criteria are determined for their separation, these species will have to be considered under the *S. dairenensis* complex. One pair of homologous strains, which can be distinguished by a few simple tests, has been designated as the new species *S. rosinii* (Vaughan-Martini et al. 1996).

44.6. *Saccharomyces exiguus* Reess ex E.C. Hansen (1888a)

Anamorph: *Candida holmii* (Jörgensen) S.A. Meyer & Yarrow

Synonyms:

Torula holmii Jörgensen (1909)

Torulopsis holmii (Jörgensen) Lodder (1934)

Cryptococcus holmii (Jörgensen) Skinner (1950)

Candida holmii (Jörgensen) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

?*Torula alactosa* Harrison (1928)

Growth in 5% malt extract: After 3 days at 25°C, the cells are subglobose to ellipsoidal, elongate, (2.5–5.0)×(3.5–6.5) µm, single or in pairs. After one month at 20°C, a sediment is present and occasionally a ring.

Growth on 5% malt agar: After one month at 20°C, the streak culture is butyrous, cream-colored to tan, smooth with light striations, flat and rather spreading, glossy, margin usually entire.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are not formed.

Formation of ascospores: Vegetative cells are transformed directly into asci containing one to two, rarely four, globose to ellipsoidal ascospores.

Sporulation was observed on acetate agar and V8 agar after 7–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	s
Sucrose	+	Trehalose	+
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	s
Sucrose	+	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	s
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	v
L-Lysine	–	1000 ppm Cycloheximide	v
Ethylamine·HCl	–	Growth at 37°C	–

Co-Q: 6 (Yamada et al. 1976b).

Mol% G+C: 30.5, AJ 4075 (T_m: Nakase and Komagata 1968a); 33.2–33.7, AJ 4075, AJ 4349 (T_m: Nakase and Komagata 1971e); 35.2, CBS 379 (T_m: Yarrow and Nakase 1975); 32.9–34.9, 10 strains (T_m: Yarrow 1984a); 34.7–36.3, CBS 134, CBS 135, CBS 397, CBS 2141 (BD: Vaughan-Martini and Kurtzman 1988).

Origin of the strains studied: DBVPG 6252 (CBS 379), unknown; DBVPG 6354 (CBS 1515), fermenting cucumber brine; DBVPG 6358 (CBS 2141), soil; DBVPG 6367 (CBS 1514), fermenting cucumber brine; DBVPG

6369 (CBS 6388), fruit of *Fragaria ananassa*; DBVPG 6402 (CBS 4660), grape must; DBVPG 6413 (CBS 134), unknown; DBVPG 6468 (CBS 8135) and DBVPG 6469 (CBS 8134), soil; DBVPG 6481 (CBS 135), type strain of *Candida holmii*, buttermilk; DBVPG 6484 (CBS 6440), sewage slick; DBVPG 6749 (CBS 4661), grape must.

Type strain: CBS 379 (ATCC 10599; DBVPG 6252; IFO 1128; NRRL Y-1538) from Naganishi, and chosen as type strain by Lodder and Kreger-van Rij (1952).

Comments: DNA reassociation studies (Vaughan-Martini and Kurtzman 1988) revealed 76% nucleotide sequence relatedness between the type strains of *S. exiguus* and *Candida holmii*, therefore confirming this teleomorphic–anamorphic relationship. On the other hand, low relatedness between these strains and others designated as *S. exiguus* led to the hypothesis that the latter are different species. The possible presence of more than one species within the group of strains commonly designated as *S. exiguus* was also supported by other workers (van der Walt 1970d, Yarrow 1978, Kaneko et al. 1989). This has been confirmed by a second study of DNA base sequence homology enlarged to a greater number of strains. Three separate groups have been identified: the main *S. exiguus* complex comprised of a number of variably related strains and two new separate species: *S. barnettii* and *S. spencerorum* (Vaughan-Martini 1995).

44.7. *Saccharomyces kluyveri* Phaff, M.W. Miller & Shifrine (1956)

Synonyms:

Saccharomyces smittii Capriotti (1958d)
Saccharomyces silvestris Jensen (1967)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ellipsoidal or cylindroidal, (3.5–7.0)×(4.0–11.0) µm, single, in pairs or in clusters (Fig. 157). After one month at 20°C, a sediment and a ring are present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is cream-colored to tan, fairly flat and smooth to raised and wrinkled or folded, often sectored, glossy or dull, margin undulating or lobate and sometimes fringed with pseudohyphae.

Growth on the surface of assimilation media: Pellicles are not formed.

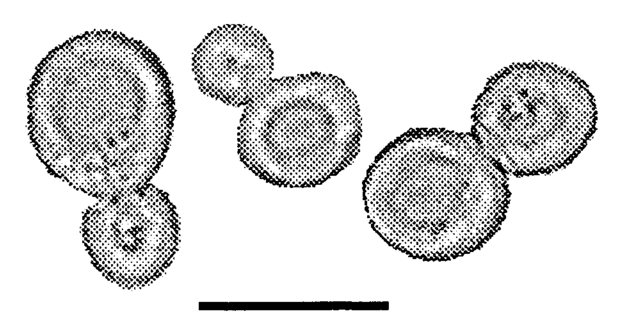


Fig. 157. *S. kluyveri*, CBS 3082. Vegetative cells after 3 days, on YEPG agar, 25°C. Bar = 10 µm.

Dalmau plate culture on morphology agar: Pseudohyphae are usually formed.

Formation of ascospores: Vegetative cells are transformed directly into asci containing one to four globose to subglobose ascospores.

Sporulation was observed on acetate agar after 4 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–	Melibiose	+

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	+	100 ppm Cycloheximide	–
L-Lysine	+	Growth at 37°C	+
Ethylamine·HCl	+		

Co-Q: Not determined.

Mol% G+C: 41.9, one strain, CBS 3082, (BD: Vaughan-Martini and Kurtzman 1985).

Origin of the strains studied: DBVPG 3103, 3108, 3109, 3452 and 3453, soil; DBVPG 6177 (CBS 3082), *Drosophila*; DBVPG 6362 (CBS 6545), slime flux; DBVPG 6363 (CBS 6626), tree exudate; DBVPG 6403 (CBS 5828), water-logged soil; DBVPG 6412 (CBS 6548), *Drosophila*; DBVPG 6430 (CBS 4104), soil; DBVPG 6444 (CBS 4570), unknown; DBVPG 6467 (CBS 4569), unknown.

Type strain: CBS 3082 (DBVPG 6177, IFO 1685, NRRL Y-12651, UCD-FST 51-242) isolated from *Drosophila pinicola* at Mather, California, U.S.A.

Comments: *Saccharomyces kluyveri* was provisionally included in group 2 of the genus *Saccharomyces sensu* van der Walt (1970d). Meyer and Phaff (1969), however, suggested that it fit better into group 1, *Saccharomyces sensu stricto*, because of its apparent close resemblance to *Saccharomyces cerevisiae*. This was in part due to the results of Barker and Miller (1969), who had observed the formation of a few infertile zygotes between haploid strains of *S. kluyveri* and *S. cerevisiae*. McCullough

and Herskowitz (1979) also noted physiological cross-reactions of pheromones between the two species.

Later, Johnston and Mortimer (1986) expressed doubts that *S. kluyveri* is a true member of the group. In fact, results of an OFAGE (orthogonal field inversion gel electrophoresis) analysis revealed that while typical strains of *Saccharomyces sensu stricto* have 12–15 variably-sized chromosomes, *S. kluyveri* has only 3 (later seen to be 5–6, Vaughan-Martini et al. 1993) large-sized (>1000 kb) chromosomes. That later study also revealed that all other species of *Saccharomyces,* with the exception of *S. dairenensis,* are characterized by the presence of at least 10 chromosomes covering all size ranges: small (<500 kb), medium (500–1000 kb), and large (>1000 kb). In addition, studies of ribosomal RNA sequences (Kurtzman and Robnett 1991) showed that *S. kluyveri* is the most divergent member of the genus.

44.8. *Saccharomyces paradoxus* Bachinskaya (1914)

Synonyms:

Zygosaccharomyces paradoxus (Bachinskaya) Klöcker (1924)
Zygosaccharomycodes paradoxus (Bachinskaya) Nishiwaki (1929)
Saccharomyces mangini Guilliermond var. *tetraspora* (Beijerinck ex Dekker) Stelling-Dekker (1931)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *tetraspora* (Beijerinck ex Dekker) Phaff, M.W. Miller & Shifrine (1956)
Saccharomyces cerevisiae Meyen ex E.C. Hansen var. *terrestris* Jensen (1967)
Saccharomyces douglasii Hawthorne & Philippsen (1994) nom. nud.

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ellipsoidal or cylindroidal, (2.5–7.0)×(3.0–8.5)µm, single, in pairs or in clusters. After one month at 20°C, a sediment and a ring are present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is cream-colored to tan, smooth to raised and dull; the margin is undulating or lobate and sometimes fringed or smooth.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are occasionally formed.

Formation of ascospores: Vegetative cells are transformed directly into asci containing one to four globose to subglobose ascospores. In most strains over 50% of the cells sporulate and ascospores are highly viable.

Sporulation, when observed, occurs on almost any medium such as malt extract agar, yeast extract, glucose agar (YEPG), potato agar and acetate agar in 2–4 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	v	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	–
L-Lysine	–	Active fructose transport	–
Ethylamine·HCl	–	Growth at 37°C	+

Co-Q: Not determined.

Mol% G+C: 42.0–43.1, CBS 432, CBS 5829, DBVPG 6304 (T_m: Vaughan-Martini, unpublished data).

Origin of the strains studied: DBVPG 6045 (CBS 406), type strain of *S. mangini* var. *tetraspora,* oak exudate; DBVPG 6303 (UCD-FST 52-153) and DBVPG 6304 (UCD-FST 51-186), *Drosophila*; DBVPG 6411 (CBS 432), tree exudate; DBVPG 6466 (CBS 5829) type strain of *S. cerevisiae* var. *terrestris,* soil; DBVPG 6487, DBVPG 6490, DBVPG 6491, DBVPG 6493 and DBVPG 6494, tree exudates.

Type strain: CBS 432 (DBVPG 6411, IFO 10609, IGC 4570, NRRL Y-17217) was designated as the neotype.

Comments: Recent ecological, taxonomic and genetic studies have led to the hypothesis that *S. paradoxus* is the natural parent species of the domesticated species of the *Saccharomyces sensu stricto* group which includes *S. bayanus, S. cerevisiae* and *S. pastorianus.* This premise is supported in part by the results of DNA reassociation studies which showed that while *S. paradoxus* and *S. cerevisiae* are related for about 50% of their nucleotide sequences, relatedness with *S. bayanus* and *S. pastorianus* is less than 20% (Vaughan-Martini 1989). In addition, Naumov (1986b, 1987c) found that effective mating does not occur between *S. paradoxus* and *S. bayanus* or with *S. cerevisiae.*

In light of the above results, and considering the ecological separation of *S. paradoxus* from the other three species (only natural sources in contrast to mostly artificial fermentation environments for the other three, Phaff et al. 1956, Rosini et al. 1982a, Rosini 1984, Naumov and Nikonenko 1988), it seems appropriate that this species be considered a separate member of the *Saccharomyces sensu stricto* complex.

44.9. *Saccharomyces pastorianus* E.C. Hansen (1904)

Synonyms:
Saccharomyces carlsbergensis E.C. Hansen (1908)
Saccharomyces monacensis E.C. Hansen (1908)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ellipsoidal or cylindroidal, (2.2–8.0)×(3.5–13.5) μm, single, in pairs or in clusters. After one month at 20°C, a sediment is present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is cream-colored to light tan, fairly flat and smooth with the margin slightly undulating or lobate and sometimes fringed or smooth.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are generally not present.

Formation of ascospores: Vegetative cells are transformed directly into asci containing one to four globose to subglobose ascospores. In most strains sporulation is less than 2% and ascospores are usually not viable.

Sporulation, when observed, occurs only on acetate agar after 10–14 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	+
Sucrose	v	Trehalose	–
Maltose	+	Melibiose	v

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	–
L-Lysine	–	Active fructose transport	+
Ethylamine·HCl	–	Growth at 37°C	–

Co-Q: 6 (Yamada et al. 1976b).

Mol% G+C: 38.5–39.5, AJ 4033, AJ 4034, AJ 4059, AJ 53137 (T_m: Nakase and Komagata 1971e); 41.1, CBS 1513, (BD: Vaughan-Martini and Kurtzman 1985); 41.2–42.0, CBS 1513, CBS 1538 (T_m: Vaughan-Martini, unpublished data).

Origin of the strains studied: DBVPG 6033 (CBS 1513), type strain of *S. carlsbergensis,* brewery; DBVPG 6047 (CBS 1538), unknown; DBVPG 6257 (CBS 1260), unknown; DBVPG 6258 (CBS 1486), beer; DBVPG 6261 (CBS 1503), type strain of *S. monacensis*, beer; DBVPG 6282 (Carlsberg BK 2233), DBVPG 6283 (Carlsberg BK 2230), DBVPG 6284 (Carlsberg AJL 248) and DBVPG 6285 (Carlsberg M 1563), all probably from beer.

Type strain: CBS 1538 (ATCC 12752, DBVPG 6047, IFO 0613, NCYC 392, NRRL Y-1551), has been designated as the type strain and is probably isolated from beer.

Comments: Some problems have been encountered distinguishing between the species *S. cerevisiae* and *S. pastorianus* since the two are phenotypically close. While the large majority of strains belonging to *S. cerevisiae* grow at temperatures up to 40°C, a sole exception was seen for the type strain of *S. oviformis,* now a synonym of *S. cerevisiae.* None of the strains of *S. pastorianus* studied were able to grow above 34°C. As a result, temperature can be a reliable criterion for the separation of strains growing above 34°C. A recent study on the ability to actively transport fructose has shown that strains belonging to *S. paradoxus* and *S. cerevisiae* are consistently characterized by a negative fructose transport system. By contrast, strains of *S. bayanus* and *S. pastorianus* actively transport fructose (Rodrigues de Sousa et al. 1990).

Extensive genetic and molecular studies of numerous brewery yeasts appear to indicate that *S. pastorianus* is the most favorable species for beer making. This is in spite of the fact that the type strains of two of the other species of the *Saccharomyces sensu stricto* complex (*S. cerevisiae* and *S. bayanus*) were originally isolated from beer.

44.10. *Saccharomyces rosinii* Vaughan-Martini, Barcaccia & Pollacci (1996)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal or elongate, (3.0–5.5)×(3.5–7.0) μm, and single or in pairs. After one month at 20°C, a sediment is present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is butyrous and light cream-colored. The surface is smooth and glossy and the margin is entire or crenate.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: No pseudohyphae are formed.

Formation of ascospores: Vegetative cells are transformed directly into persistent asci containing one to two globose ascospores.

Very limited sporulation was observed with asci containing one or two globose ascospores. Sporulation was obtained after 9–14 days on acetate agar at 20°C or in YEPG at 5°C for 20–25 days.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	–
L-Lysine	–	Growth at 37°C	–
Ethylamine·HCl	–		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: DBVPG 6747 (CBS 7127) and DBVPG 6750 (CBS 7128), both of unknown origin.

Type strain: CBS 7127 (DBVPG 6747).

Comments: See Comments under *S. dairenensis.*

44.11. *Saccharomyces servazzii* Capriotti (1967)

Growth in 5% malt extract: After 3 days at 25°C, the cells are subglobose to ellipsoidal, (3.0–4.5)×(4.0–5.5) µm, and single or in pairs. After one month at 20°C, a sediment and a thin ring are present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is tan, flat, smooth with faint striations, waxy and glossy; the margin is slightly crenate.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are not formed.

Formation of ascospores: Vegetative cells are transformed directly into asci containing one to four globose to short ellipsoidal ascospores.

Sporulation was observed on acetate agar after 7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm Cycloheximide	+
L-Lysine	–	1000 ppm Cycloheximide	+
Ethylamine·HCl	–	Growth at 37°C	–

Co-Q: Not determined.
Mol% G+C: 33.9, CBS 4311 (T_m: Yarrow and Nakase 1975); 34.4–35.9, 2 strains (T_m: Yarrow 1984a); 36.6, CBS 4311 (BD: Vaughan-Martini and Kurtzman 1988).

Origin of the strains studied: DBVPG 6355 (CBS 4311), from soil; DBVPG 6360 (CBS 6865) of unknown origin.

Type strain: CBS 4311 (DBVPG 6355, NRRL Y-12661). Since a type was not designated for this species by Capriotti (1967), one of the isotypes was chosen by Yarrow (1984a) as the lectotype.

Comments: See Comments under *S. unisporus.*

44.12. *Saccharomyces spencerorum* Vaughan-Martini (1995)

Growth in 5% malt extract: After 3 days at 25°C, the cells are subglobose to ellipsoidal, elongate, (2.5–5.0)×(3.5–6.5) µm, single or in pairs. After one month at 20°C, a sediment is present and often also a ring and a film.

Growth on 5% malt agar: After one month at 20°C, the streak culture is butyrous, cream-colored to tan, smooth with light striations, flat and rather spreading, glossy, and the margin usually entire.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are not formed.

Formation of ascospores: Vegetative cells are transformed directly into asci containing one or two, rarely four, globose to ellipsoidal ascospores.

Sporulation was observed on acetate agar and V8 agar after 7–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	s	Trehalose	+
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	s
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	+	100 ppm Cycloheximide	–
L-Lysine	+	Growth at 37°C	+
Ethylamine·HCl	+		

Co-Q: Not determined.
Mol% G+C: 34.4–35.1, CBS 3019, CBS 5530 (T_m: Yarrow 1978).

Origin of the strains studied: DBVPG 6746 (CBS 3019), soil; DBVPG 6748 (CBS 5530), gut of *Psychidae* larva on *Acacia hereocantha.*

Type strain: CBS 3019 (DBVPG 6746), from soil.

Comments: Thiamine is required for growth (Yarrow, personal communication). For additional information see the Comments under *S. exiguus.*

44.13. *Saccharomyces transvaalensis* van der Walt (1956c)

Synonym:
Pachytichospora transvaalensis (van der Walt) van der Walt (1978)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ovoidal or elongate, (3.0–5.5)×(3.5–7.0) μm, single or in pairs. After one month at 20°C, a sediment is present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is butyrous and light cream-colored. The surface is smooth and glossy and the margin is entire or crenate.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: No pseudohyphae are formed.

Formation of ascospores: Vegetative cells are transformed directly into persistent asci containing one or two large, highly refringent, globose ascospores.

Sporulation was observed on acetate agar and YM agar in some strains in 7–9 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	v	100 ppm Cycloheximide	–
L-Lysine	v	Growth at 37°C	+
Ethylamine·HCl	–		

Co-Q: 6 (Yamada et al. 1976b).
Mol% G+C: 32.0, CBS 2186 (Yarrow & Nakase 1975); 34.1–34.4, 3 strains (von Arx 1977b, 1978).

Origin of the strains studied: DBVPG 6756 (CBS 7662) and DBVPG 6757 (CBS 2186), soil.

Type strain: CBS 2186 (DBVPG 6757), isolated by van der Walt from soil in Mozambique.

Comments: Although the species *transvaalensis* was originally classified under the genus *Saccharomyces* (van der Walt 1956c), a subsequent study showed an absence of interfertility between mating types of *S. transvaalensis* and two strains classified as the most phenotypically similar species, *S. dairenensis* (van der Walt and Liebenberg 1973b). This prompted van der Walt (1978) to create the genus *Pachytichospora* to accommodate *S. transvaalensis* with its distinctive ascospores, which upon examination by transmission electron microscopy (TEM), appeared different from those of the type strain of *S. dairenensis* (van der Walt and Liebenberg 1973b). A recent study of DNA sequence relatedness (Vaughan-Martini and Barcaccia 1996) has demonstrated that while two of the four known strains of *S. transvaalensis* are homologous

to the type strain of *S. castellii,* the other two show an intermediate relationship with both that species and the type strain of *S. dairenensis.* In view of this intermediate position between two unrelated taxa, it was deemed appropriate to reestablish the species *S. transvaalensis* (Vaughan-Martini and Pollacci 1996). This separation is supported by the finding that interspecific rRNA sequence differences between the type strains of *S. castellii, S. dairenensis* and *S. transvaalensis* averaged 10% (Kurtzman, personal communication). The same study also noted no intraspecific variation in 600 nucleotides of rRNA between the same strains, which should further justify the abolition of the genus *Pachytichospora.* It should be recognized that it is difficult to clearly separate the taxa *S. castelli, S. dairenensis* and *S. transvaalensis* solely on the basis of conventional taxonomic criteria. A definite resolution is possible with the use of techniques of molecular taxonomy such as nucleotide base sequence relatedness as determined by DNA/DNA reassociation experiments, examination of comparable rRNA sequences, or the determination of electrophoretic karyotypes obtained by PFGE (pulsed field gel electrophoresis).

44.14. *Saccharomyces unisporus* Jörgensen (1909)

Synonym:
Saccharomyces mongolicus Naganishi (1928)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose to short ellipsoidal, (2.5–4.5)×(3.0–6.0) µm, and single or in pairs. After one month at 20°C, a sediment and a ring are present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is cream-colored, flat, usually glossy, smooth, sometimes with light striations, and the margin is entire or undulating.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: Pseudohyphae are not formed.

Formation of ascospores: Vegetative cells are transformed directly into asci containing one, occasionally two, globose to subglobose ascospores.

Sporulation was observed on acetate agar and YM agar after 7–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	s
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	+	100 ppm Cycloheximide	+
L-Lysine	+	1000 ppm Cycloheximide	+
Ethylamine·HCl	+	Growth at 37°C	v

Co-Q: 6 (Yamada et al. 1976b).
Mol% G+C: 32.4–32.7, AJ 4085, AJ 5248, AJ 5253 (T_m: Nakase and Komagata 1971e); 35.4–35.7, DBVPG 6368 (CBS 398), DBVPG 6414 (CBS 399) (BD: Vaughan-Martini and Kurtzman 1988)

Origin of the strains studied: DBVPG 6368 (CBS 398), unknown; DBVPG 6414 (CBS 399), naichin; DBVPG 6429 (CBS 2422), kefyr grains; DBVPG 6445 (CBS 3004), feces; DBVPG 6482 (CBS 1543), unknown; DBVPG 6483 (CBS 1575), cheese.

Type strain: CBS 398 (ATCC 10612, DBVPG 6368, IFO 0316, NRRL Y-1556), received from the Carlsberg laboratories in 1924, was designated as the type strain.

Comments: Even though Yarrow and Nakase (1975) noted that *S. unisporus* has many phenotypic similarities with the other species of *Saccharomyces sensu lato* (*sensu* van der Walt 1970d) i.e., *S. castellii, S. dairenensis* and *S. servazzii, S. unisporus* is easily distinguishable by having predominantly one-spored asci, as well as by the ability to grow on ethylamine, cadaverine and L-lysine as sole nitrogen sources. The results of a DNA reassociation study (Vaughan-Martini and Kurtzman 1988) supported the retention of these taxa as separate biological species since nucleotide sequence similarities between them were never greater than 19%.

Comments on the species included in the complex *Saccharomyces sensu stricto*

The collective group of species denominated as *Saccharomyces cerevisiae* (*sensu* Yarrow 1984a) includes the most industrially exploited microorganisms known to man as well as the organism of choice as a model system of the eukaryotic cell (Mortimer and Schild 1985, Silverman 1987, Clark-Walker 1989). The variable physiological behavior often observed for *Saccharomyces sensu stricto* (Scheda and Yarrow 1966, 1968) has resulted over the

years in the proposal of innumerable new epithets for conspecific strains. Genetic analysis has shown that the variable phenotypic expression exhibited by these strains is the result of the presence of multiple gene loci (Carlson 1987), which can be active, silent or missing, rather than because of the existence of genetically distinct species. It has also been demonstrated that these species, for the most part domesticated as a result of millennia of exploitation in man-created environments, are often polyploid or aneuploid. This explains why conventional mating as a tool for genetic study and strain improvement has been arduous and also why definite identification of a strain of *S. cerevisiae* or some other related species is often an almost impossible task.

A series of DNA hybridization analyses (Rosini et al. 1982b, Vaughan-Martini and Kurtzman 1985, Vaughan-Martini and Martini 1987a, 1993, Vaughan-Martini 1989), as well as traditional genetic studies (Naumov 1986b, Banno and Kaneko 1989) have shown that *Saccharomyces sensu stricto* includes four variably related species: *S. bayanus, S. cerevisiae, S. paradoxus* and *S. pastorianus.* Intermediate values of nucleotide sequence homology have been found between some species of this complex: 72% between *S. bayanus* and *S. pastorianus* and about 50% between *S. cerevisiae* and both *S. pastorianus* and *S. paradoxus.* Low nucleotide sequence homology was seen between *S. bayanus* and *S. cerevisiae* and between *S. paradoxus* and *S. bayanus* or *S. pastorianus.* These results were in part confirmed by studies of chromosomal and gene loci polymorphisms (Holmberg 1982, Johnston and Mortimer 1986, Johnston et al. 1988, Nilsson-Tillgren et al. 1981, 1983, 1986, Pedersen 1983, 1986). Later studies of ribosomal RNA sequence divergence (Kurtzman and Robnett 1991) have further supported the hypothesis that this is a group of taxa in the early stages of the speciation process.

Note in proof

James et al. (1997) analyzed relationships among *Saccharomyces* species from divergence in 18S rDNA. Included in this study was the description of two new species of *Saccharomyces*: *S. kunashirensis* James, Cai, Roberts & Collins and *S. martiniae* James, Cai, Roberts & Collins.

45. *Saccharomycodes* E.C. Hansen

M.W. Miller and H.J. Phaff

Diagnosis of the genus

Large, diploid, lemon-shaped (apiculate) or elongate cells, which reproduce by bipolar budding on a very broad base (bud-fission). Pseudomycelium is absent or poorly developed.

The spores are spheroidal with an apparently smooth wall; a narrow subequatorial ledge and irregularly spaced surface nodules may be visible. Asci usually contain four spores, occurring in two pairs; upon germination members of each pair ordinarily conjugate. In some strains spores may germinate without conjugation.

Sugars are fermented. Nitrate is not assimilated. In liquid media a sediment and a ring are formed. Diazonium blue B reaction is negative.

Type species

Saccharomycodes ludwigii E.C. Hansen

Species accepted

1. *Saccharomycodes ludwigii* E.C. Hansen (1904)

Systematic discussion of the species

45.1. *Saccharomycodes ludwigii* E.C. Hansen (1904)

Synonyms:
Saccharomyces ludwigii E.C. Hansen (1889)
Saccharomycodes ludwigii (E.C. Hansen) E.C. Hansen var. *vini* Kroemer & Heinrich (1922)
Saccharomycodes vini (Kroemer & Heinrich) ex Kudryavtsev (1960)
Saccharomycodes bisporus Castelli (1942)
Saccharomycodes ludwigii (E.C. Hansen) E.C. Hansen var. *bisporus* (Castelli) Hjort (1954)
Saccharomycodes lipophora Bachinskaya (1941)
Saënkia bispora Castelli ex Kudryavtsev (1960)

Growth in 5% malt extract: After 3 days at 25°C, the cells are lemon-shaped with blunt tips, sausage-shaped, curved, broad-oval, or elongated with a swelling in the middle, (4–7)×(8–23) µm, and occasionally longer; the cells are single or in pairs and sometimes in groups of three. Reproduction occurs by bipolar budding on a broad base, followed by fission (bud-fission). Cells in the process of budding resemble bowling pins (Fig. 158). A sediment and a slight ring are present. After approximately one month, a sediment and a ring are in evidence.

Growth on 10% malt agar: After 3 weeks the streak culture is cream-colored to tan, nearly smooth, semi-glossy, and low convex with an irregular border.

Dalmau plate culture on potato agar: Aerobically there are a few short chains of cells. Under the coverslip the pseudomycelium is somewhat better developed, consisting of branched chains of elongated cells.

Formation of ascospores: The spores are spheroidal, smooth with a narrow subequatorial ledge which is visible in sections viewed in the electron microscope (Kreger-van Rij 1969b); in freeze-fracture replicas the outer surface of the spores shows irregularly spaced nodules (Simmons and Ahearn 1985); usually there are four spores per ascus in two groups of two (Fig. 159). Some strains produce two spores per ascus. Ascus walls do not rupture or lyse when the spores are mature. For germination of spores see Diagnosis of the genus.

Good sporulation was obtained on Gorodkowa agar, in 3 days at 18°C.

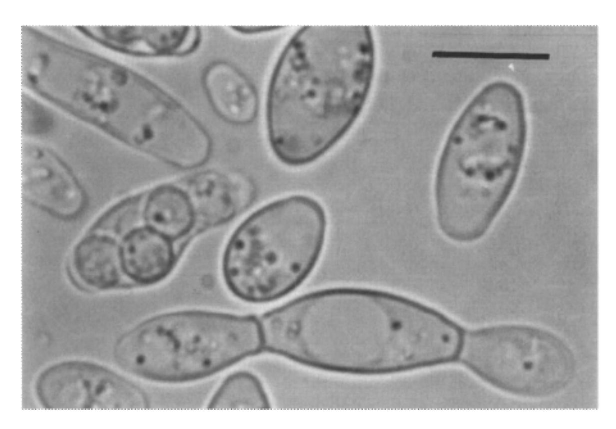


Fig. 158. *S. ludwigii*, CBS 821. Vegetative cells grown on 10% malt agar after 3 days. Bar = 10 µm.

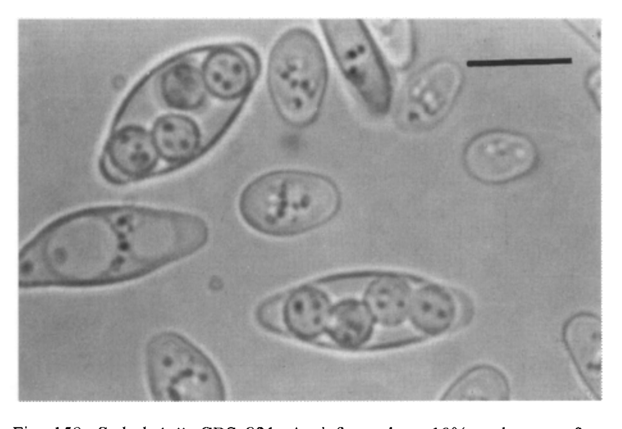


Fig. 159. *S. ludwigii*, CBS 821. Asci formed on 10% malt agar after 3 days. Bar = 10 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	w
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	l
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	–
5-Keto-D-gluconate	–	Lipase	–
Cadaverine	+	Gelatin hydrolysis	w
L-Lysine	l	Cycloheximide 1 μg/ml	+
Ethylamine	+	Cycloheximide 10 μg/ml	–
50% Glucose	–	Acid production	–
5% NaCl/5% glucose	–	Growth at 30°C	+
Starch synthesis	–	Growth at 37°C	–

Co-Q: 6, CBS 821 and 5 additional strains (Yamada et al. 1976a).

Mol% G+C: 38.3, strain AJ 5323 from the Central Research Laboratories of Ajinomoto Co., Inc., Japan (Nakase and Komagata 1971g).

Origin of the strains studied: CBS 821, diploid strain from A.J. Kluyver; CBS 2624, type strain of *Saccharomycodes bisporus* Castelli; 2 diploids, 3 short-celled haploids, 3 long-celled haploids from Carlsberg Laboratory in Copenhagen; UCD-FST 72-128, exudate of *Quercus gambelii*, Arizona; UCD-FST 77-13, exudate from swamp chestnut oak, *Quercus prinus* (=*Q. michauxii*), Virginia, U.S.A.

Type strain: CBS 821 (ATCC 11313), origin M.W. Beijerinck.

Species not accepted in the genus

We did not accept *Saccharomycodes sinensis* Yue (1977) because its characteristics are very different from those of *Saccharomycodes* E.C. Hansen. It does not assimilate ammonium sulfate as a sole source of nitrogen but requires a complex mixture of amino acids as is present in yeast autolyzate. Although the yeast reproduces by a kind of bud fission, the cells are only about one fourth as long as those of *S. ludwigii*. We were unable to detect ascospore formation on Gorodkowa agar or on Kleyn's acetate agar as recommended by Yue (1977). We believe that further studies are required to place this species in an appropriate genus.

Note in proof

The nucleotide sequences for *Saccharomycodes sinensis* and *Nadsonia fulvescens* var. *elongata* are identical in the variable 600-nucleotide 5′ end of the large subunit rRNA gene, suggesting that the two taxa are conspecific (Kurtzman and Robnett, manuscript in preparation).

46. *Saccharomycopsis* Schiönning

C.P. Kurtzman and M.Th. Smith

Diagnosis of the genus

Abundant development of true mycelium, often with blastoconidia. Budding cells are present and may be formed on a broad or a narrow base. Arthroconidia are produced by some species. Hyphal septa are usually multiperforate, but may be limited to a single central pore.

Asci are usually attached to hyphae and produce 1–4, or rarely 8, ascospores. Ascospores may be hat-shaped, reniform with terminal appendages, spheroidal or ellipsoidal, and may have one or more ledges; surfaces may be smooth or roughened.

Some species are fermentative. Nitrate is not assimilated. Coenzyme Q-8 is the predominant ubiquinone. Diazonium blue B reaction is negative.

Type species

Saccharomycopsis capsularis Schiönning

Species accepted

1. *Saccharomycopsis capsularis* Schiönning (1903)
2. *Saccharomycopsis crataegensis* Kurtzman & Wickerham (1973)
3. *Saccharomycopsis fermentans* (C.-F. Lee, F.-L. Lee, Hsu & Phaff) Kurtzman & Robnett (1995)
4. *Saccharomycopsis fibuligera* (Lindner) Klöcker (1924)
5. *Saccharomycopsis javanensis* (Klöcker) Kurtzman & Robnett (1995)
6. *Saccharomycopsis malanga* (Dwidjoseputro) Kurtzman, Vesonder & Smiley (1974)
7. *Saccharomycopsis schoenii* (Nadson & Krasil'nikov) Kurtzman & Robnett (1995)
8. *Saccharomycopsis selenospora* (Nadson & Krasil'nikov) Kurtzman & Robnett (1995)
9. *Saccharomycopsis synnaedendra* D.B. Scott & van der Walt (1971)
10. *Saccharomycopsis vini* (Kreger-van Rij) van der Walt & D.B. Scott (1971)

Key to species

See Table 38.

1.	a	Galactose assimilated	*S. selenospora*:	p. 381
	b	Galactose not assimilated → 2		
2(1).	a	L-Rhamnose assimilated	*S. synnaedendra*:	p. 382
	b	L-Rhamnose not assimilated → 3		
3(2).	a	Cellobiose assimilated → 4		
	b	Cellobiose not assimilated → 6		
4(3).	a	Inositol weakly or strongly assimilated	*S. fibuligera*:	p. 377
	b	Inositol not assimilated → 5		
5(4).	a	Growth at 37°C	*S. malanga*:	p. 379
	b	Absence of growth at 37°C	*S. capsularis*:	p. 375
6(3).	a	Glucitol assimilated → 7		
	b	Glucitol not assimilated → 8		
7(6).	a	D-Gluconate assimilated	*S. crataegensis*:	p. 376
	b	D-Gluconate not assimilated	*S. vini*:	p. 383
8(6).	a	Glucose fermented	*S. fermentans*:	p. 377
	b	Glucose not fermented → 9		
9(8).	a	*N*-Acetyl-D-glucosamine assimilated	*S. javanensis*:	p. 379
	b	*N*-Acetyl-D-glucosamine not assimilated	*S. schoenii*:	p. 380

Table 38
Key characters of species assigned to the genus *Saccharomycopsis*

Species	Glucose fermentation	Assimilation							Growth at 37°C
		Galactose	L-Rhamnose	Cellobiose	*N*-Acetyl-D-glucosamine	Glucitol	Gluconate	Inositol	
Saccharomycopsis capsularis	+	–	–	+	–	+	+	–	–
S. crataegensis	w/–	–	–	–	v	+	+	–	–
S. fermentans	+	–	–	–	–	–	–	–	–
S. fibuligera	+/w	–	–	+	–	v	+/w	+/w	+/w
S. javanensis	–	–	–	–	+	–	–	–	–
S. malanga	w	–	–	+	–	+	+	–	+
S. schoenii	–	–	–	–	–	–	–	–	–
S. selenospora	+/w	+	–	–	–	–	v	–	–
S. synnaedendra	–	–	+	–	–	+	+	+	–
S. vini	+	–	–	–	–	+	–	–	–

Systematic discussion of the species

46.1. *Saccharomycopsis capsularis* Schiönning (1903)

Synonyms:
Endomyces capsularis (Schiönning) Guilliermond (1909)
Williopsis capsularis (Schiönning) Zender (1925b)
Endomycopsis capsularis (Schiönning) Dekker (Stelling-Dekker 1931)
Prosaccharomyces capsularis (Schiönning) Novák & Zsolt (1961)

Growth on 5% malt extract agar: After 3 days at 25°C, budding cells are spheroidal, ellipsoidal or elongate, (3.0–11.1)×(7.0–15.8) µm, and single or in pairs. Growth is dull, tannish-white and butyrous to mycelial.

Growth on the surface of assimilation media: Pellicles are formed by some isolates.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, but sparingly branched true hyphae and infrequent pseudohyphae. Aerobic growth is tannish-white, dull, and mycelial with surface striations. Colonies are low convex with smooth to broadly lobed margins. A mildly acidic odor is present.

Formation of ascospores: Asci may form terminally on the tips of hyphae or they may be intercalary following conversion of a hyphal cell to an ascus (Fig. 160). Each ascus produces two to eight spheroidal ascospores that usually have a subequatorial ring. Asci become deliquescent at maturity. Kreger-van Rij (1970b) reported that single-ascospore isolates produce ascosporogenous colonies indicating the species to be homothallic.

Ascospores were observed on YM and Difco yeast morphology agars after 5–10 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	+/w		

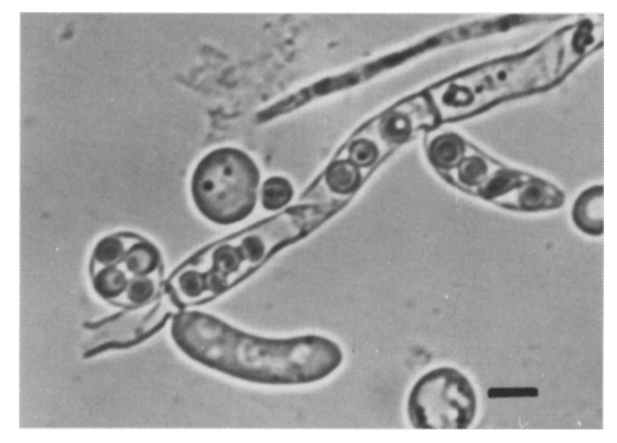


Fig. 160. *S. capsularis*, CBS 2519. Asci with ascospores. Asci may arise from hyphae or hyphal cells may be converted to asci. On YM agar, after 12 days, 25°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+/w
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	w/-
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	w
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	+/w		

Co-Q: 8, CBS 5063 (Yamada et al. 1976b).
Mol% G+C: 43.4, CBS 5063 (T_m: Nakase and Komagata 1971g).

Origin of the strains studied: CBS 2519 (NRRL Y-17639) originally from H. Schnegg, Germany, substrate unknown; CBS 5063 (NRRL Y-7486), Harden's strain 127 originally from the National Collection of Type Cultures (UK), but received by CBS from the University of Hiroshima; CBS 5638 (NRRL Y-7487), from pollen of *Xylocopa caffra,* South Africa, J.P. van der Walt.

Type strain: CBS 2519, designated as neotype by Lodder and Kreger-van Rij (1952).

46.2. *Saccharomycopsis crataegensis* Kurtzman & Wickerham (1973)

Synonym:
Endomycopsella crataegensis (Kurtzman & Wickerham) von Arx (van der Walt and von Arx 1980)

Growth on 5% malt extract agar: After 3 days at 25°C, budding cells are ellipsoidal to elongate and frequently tapered on one end. The cells measure (2.0–3.0)×(4.0–9.5) μm, and often occur singly. Growth is dull and sometimes powdery, tannish-white and butyrous to mycelial.

Growth on the surface of assimilation media: Heavy pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant true hyphae with blastoconidia. Pseudohyphae are less frequent. Aerobic growth is grayish-white, dull, powdery and mycelial. Single colonies are low convex with entire to broadly lobed margins. A strong, fragrant odor is produced.

Formation of ascospores: This species is heterothallic and all known strains have been isolated as haploid mating types. Conjugation begins within 12 h following mixing of complementary mating types and ascospores may be observed as early as 24 h after pairing. Asci are ellipsoidal and either borne directly on hyphae or supported by short stalks. Occasionally, chains of two to four asci may be formed. Asci form two ascopores and about half of the asci eventually deliquesce. Ascospores are elongated, usually slightly flattened, have a roughened surface, and a median longitudinal ledge (Fig. 161).

Ascospores were observed on YM and 5% malt extract agars after 1–7 days at 25°C.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	v
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+/w
D-Xylose	+	Succinate	+/w
L-Arabinose	w/–	Citrate	–
D-Arabinose	w/–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+/w	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 39.1, CBS 6447 (BD: Kurtzman, unpublished).

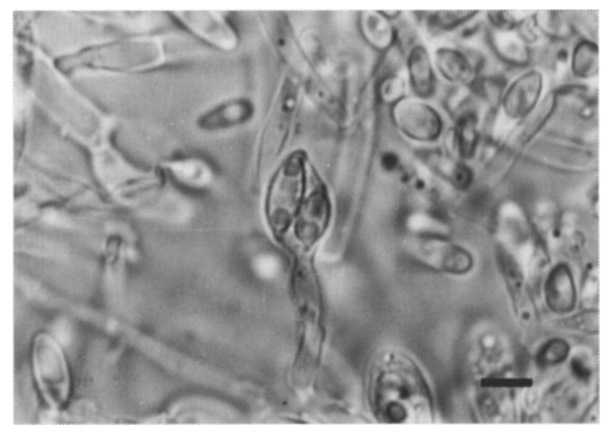


Fig. 161. *S. crataegensis,* CBS 6447×CBS 6448. Ascus with ascospores. On YM agar, after 2 weeks, 25°C. Bar = 5 μm.

Origin of the strains studied: NRRL YB-192, Concord grapes (*Vitis labrusca*), Peoria, Illinois; NRRL Y-5902 (CBS 6447), NRRL Y-5903, NRRL Y-5904 (CBS 6448), NRRL Y-5910, from fallen hawthorne (*Crataegus* sp.) fruits, Peoria, Illinois.

Complementary mating types: NRRL YB-192, NRRL Y-5902, mating type *a*; NRRL Y-5903, NRRL Y-5904, NRRL Y-5910, mating type α.

Type strain: CBS 6447, from fallen hawthorne fruits.

Comments: *S. crataegensis* and *S. vini* have similar habitats and co-occur on the fruits of grapes. Shepherd et al. (1987) and Bolen et al. (1992) reported some strains of *S. crataegenis* to contain linear DNA plasmids and/or double-stranded RNA plasmid-like molecules.

46.3. *Saccharomycopsis fermentans* (C.-F. Lee, F.-L. Lee, Hsu & Phaff) Kurtzman & Robnett (1995)

Synonym:

Arthroascus fermentans C.-F. Lee, F.-L. Lee, Hsu & Phaff (1994a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal or elongate, (2.5–5.0)×(5.0–17.3) μm, and often tapered. The cells are mostly single. Budding may be on either a narrow or rather broad base. Growth is somewhat butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, moderately branched true hyphae, but with few blastoconidia. Pseudohyphae are infrequent. Aerobic growth is tannish-white, dull, and low with fine striations. Margins are finely lobed with a narrow band of subaerial hyphae. A faint odor of esters is present.

Formation of ascospores: Asci are spindle-shaped and form directly from single cells or following conjugation between two independent cells. Asci become deliquescent at maturity. Each ascus forms two to four ascospores that are oblate-ovoidal and with a narrow equatorial ridge (Fig. 162). Scanning electron microscopy suggests some roughening of spore surfaces (Lee et al. 1994a). Heat treatment of ascosporogenous cultures indicates the species to be homothallic (Lee et al. 1994a).

Ascospores were observed on 5% malt extract, YM, and GPY agars after 3–7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

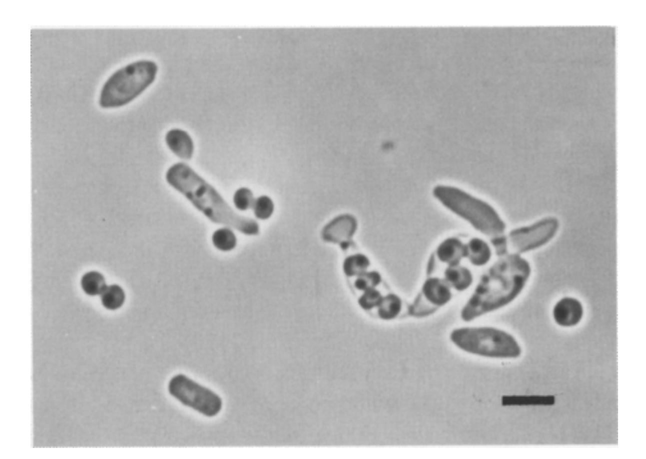


Fig. 162. *S. fermentans*, CBS 7830. Asci with ascospores. After 1 week on 5% malt extract agar, 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8, CBS 7830, 2 additional strains (Lee et al. 1994a).

Mol% G + C: 36.5–37.5, CBS 7830, CBS 8731, CBS 8732 (HPLC: Lee et al. 1994a).

Origin of the strains studied: CBS 7830 (CCRC 22530, NRRL Y-17710), soil from a papaya orchard, Taiwan; CBS 7831, CBS 7832, soil from carambola orchards, Taiwan.

Type strain: CBS 7830.

Comments: In the original description, *S. fermentans* was assigned to the genus *Arthroascus*, but rDNA sequence comparisons showed *Arthroascus* to be a synonym of *Saccharomycopsis* (Kurtzman and Robnett 1995). Comparisons of nDNA complementarity showed ca. 30% relatedness between *A. fermentans* and *A. javanensis*, thus demonstrating the two taxa to be sibling species (Lee et al. 1994a).

46.4. *Saccharomycopsis fibuligera* (Lindner) Klöcker (1924)

Synonyms:

Endomyces fibuliger Lindner (1907)
Endomycopsis fibuliger (Lindner) Dekker (Stelling-Dekker 1931)
Pichia fibuliger (Lindner) Boidin, Pignal, Lehodey, Vey & Abadie (1964)
Endomyces lindneri Saito (1913)
Saccharomycopsis lindneri (Saito) Klöcker (1924)
Endomycopsis fibuliger (Lindner) Dekker var. *lindneri* (Saito) Dekker (Stelling-Dekker 1931)
Endomyces hordei Saito (1914)
Saccharomycopsis hordei (Saito) Klöcker (1924)
Endomycopsis fibuliger (Lindner) Dekker var. *hordei* (Saito) Dekker (Stelling-Dekker 1931)
Candida lactosa Dwidjoseputro & Wolf (1970)
?*Endomycopsis bubodii* Sakai & Caldo (1985) nom. nud.
Botryoascus cladosporoides Martínez, González, Abarca & Cabañes (1990)

Growth on 5% malt extract agar: After 3 days at 25°C, budding cells are ovoidal to elongate, (2.2–5.1)×(3.3–12.5) µm, sometimes tapered, and occur singly, or less frequently in pairs. Growth is dull white, sometimes powdery, and mycelial. Some strains are noticeably more mycelial than other strains.

Growth on the surface of assimilation media: Pellicles are formed by some strains.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant true hyphae with varying numbers of blastoconidia, as well as some pseudohyphae. Aerobic growth is dull and gray-white with a somewhat raised center. Tufts of hyphal outgrowths on the colony surface give a diagnostic roughness that generally allows immediate species identification. A pleasant ester-like odor is produced.

Formation of ascospores: Asci are spheroidal to ovoidal and may be free, having formed from a single vegetative cell, or attached to the ends or sides of hyphae. Occasionally, asci arise on protuberances from two adjacent hyphal cells, but generally asci are unconjugated. Each ascus forms two to four hat-shaped ascospores that are liberated at maturity (Fig. 163). Kreger-van Rij (1970b) reported that single-spore isolates form ascosporogenous colonies, indicating the species to be homothallic.

Ascospores were observed on YM, 5% malt extract, V8 or potato-dextrose agars after 3–30 days at 25°C.

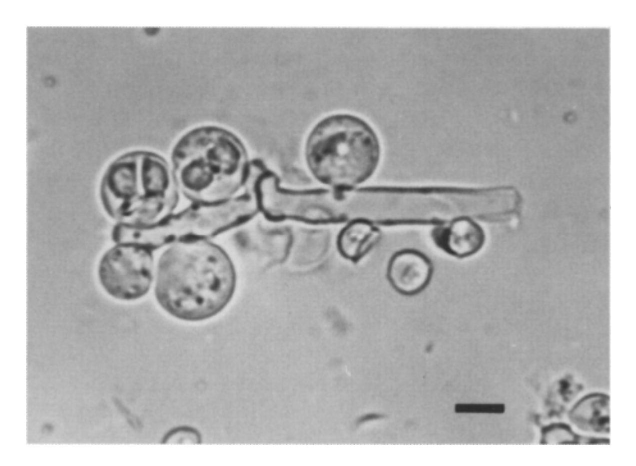


Fig. 163. *S. fibuligera*, CBS 6266. Asci and ascospores. After 2 days on potato-dextrose agar at 25°C. A curved crystal of 2-D-hydroxy fatty acids is located to the left of the scale bar. Bar = 5 µm.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	–	Raffinose	w/–
Sucrose	+/w	Trehalose	–
Maltose	+/w		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	v
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+/w
Soluble starch	+	DL-Lactate	+/w
D-Xylose	–	Succinate	+/w
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	+/w
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	w/–	Gelatin liquefaction	+/w
Saccharate	–	Growth at 37°C	+/w
10% NaCl/5% glucose	v		

Co-Q: 8, AJ 4270 (Yamada et al. 1976b).
Mol% G+C: 39.3, AJ 4270 (T_m: Nakase and Komagata 1970b).

Origin of the strains studied: Wheat flour, U.S.A. (6); chalky bread, U.S.A., Europe (4); yeast cake, U.S.A., Asia (4); macaroni, U.S.A. (9); ensiled maize, U.S.A. (2); beer starter, Tibet (3); lao chao, China (11); murcha, Nepal (17); wine koji, Korea (1); thumba, India (1); ragi, Indonesia (17); bubod, Philippines (13); chica, Bolivia (2); unknown (16).

Type strain: CBS 2521 (NRRL Y-2388), from chalky rye bread, Germany.

Comments: *S. fibuligera* is found worldwide in starchy substrates and is the major amylolytic yeast in indigenous food fermentations using rice and cassava (Hesseltine and Kurtzman 1990). Wickerham et al. (1944) were the first to report starch hydrolysis by *S. fibuligera*, and this discovery formed the basis of the Swedish Symba yeast process in which a mixed culture of *S. fibuligera* and *Candida utilis* is used to break down potato processing wastes to produce yeast cells for cattle feed (Jarl 1969).

Cultures of *S. fibuligera* commonly exhibit aggregates of crystals when viewed under the light microscope. Kurtzman et al. (1973b) determined that the crystals are comprised of mixtures of C_{14}–C_{18} 2-D-hydroxy fatty acids. Similar crystalline mixtures are found in cultures of *Pichia sydowiorum* (Vesonder et al. 1970).

In the current treatment, *Endomycopsis bubodii* is given as a possible synonym of *S. fibuligera*. Cultures of *E. bubodii* are unavailable, but the description of this species (Sakai and Caldo 1985) suggests that it may be conspecific with *S. fibuligera*.

46.5. *Saccharomycopsis javanensis* (Klöcker) Kurtzman & Robnett (1995)

Synonyms:

Endomyces javanensis Klöcker (1909b)
Schwanniomyces javanensis (Klöcker) Zender (1925b)
Endomycopsis javanensis (Klöcker) Dekker (Stelling-Dekker 1931)
Schizosaccharomyces javanensis (Klöcker) Streiblová (1963)
Arthroascus javanensis (Klöcker) von Arx (1972)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are elongated and often tapered, (2.5–5.0)×(7.4–25.0) µm, and occur singly or in pairs. Budding is generally on a broad base. Growth is tannish-white and sparingly butyrous.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows moderately branched true hyphae which are devoid of blastoconidia. Pseudohyphae are not present. Aerobic growth is somewhat butyrous, white, semi-glistening, and low convex with a depressed center. Margins are finely lobed. A faint acidic odor is present.

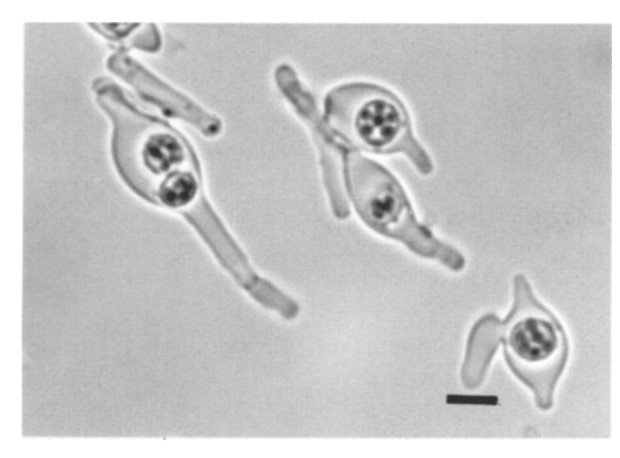


Fig. 164. *S. javanensis*, CBS 2555. Asci with ascospores. After 2 days on 5% malt extract agar at 25°C. Bar = 5 µm.

Formation of ascospores: Ascus formation is generally preceded by conjugation which may occur between independent cells or between adjacent cells following dissolution of the cross wall (Kreger-van Rij and Veenhuis 1976a). One to four ascopores form in each ascus, and asci often become spindle-shaped (Fig. 164). The ascospores, which are released at maturity, are often warty and either spheroidal or subspheroidal with an equatorial or subequatorial ledge.

Ascosporulation was observed on 5% malt extract, V8 and YM agars after 3–7 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8, CBS 2555 (Yamada et al. 1976b).
Mol% G + C: 31.5, 31.0, CBS 2555 (T_m: Nakase and Komagata 1971g, Smith et al. 1990a).

Origin of the strains studied: CBS 2555 (NRRL Y-1483), from Guilliermond and attributed to be Klöcker's original strain from soil, Java.

Type strain: CBS 2555.

46.6. *Saccharomycopsis malanga* (Dwidjoseputro) Kurtzman, Vesonder & Smiley (1974)

Synonym:

Hansenula malanga Dwidjoseputro (Dwidjoseputro and Wolf 1970)

Growth on 5% malt extract agar: After 3 days at 25°C, budding cells are mostly single, and spheroidal, (3.2–6.0) µm, or ellipsoidal to elongate, (3.0–6.0)×(8.0–13.0) µm, with many of the elongated cells somewhat tapered. Growth is dull, tannish-white, and often has a somewhat wet appearance.

Growth on the surface of assimilation media: Pellicles are formed by some strains.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, but sparingly branched true hyphae as well as pseudohyphae. Aerobic growth is dull tannish-white, and often appears somewhat wet. Colonies are low convex, striated, and margins are lobed and fringed with hyphae. A faintly acidic odor is present.

Formation of ascospores: Asci are spheroidal and may be free, but are usually borne laterally or terminally on hyphae, and may be single or in clusters of up to ten. Ascospores are hat-shaped (Fig. 165). Usually two are formed in each ascus and they are released at maturity. Single-spore isolates gave ascosporogenous

colonies indicating that either the species is homothallic or that the spores are diploid.

Ascosporulation was observed on YM and 5% malt extract agars after 3–7 days at 25°C.

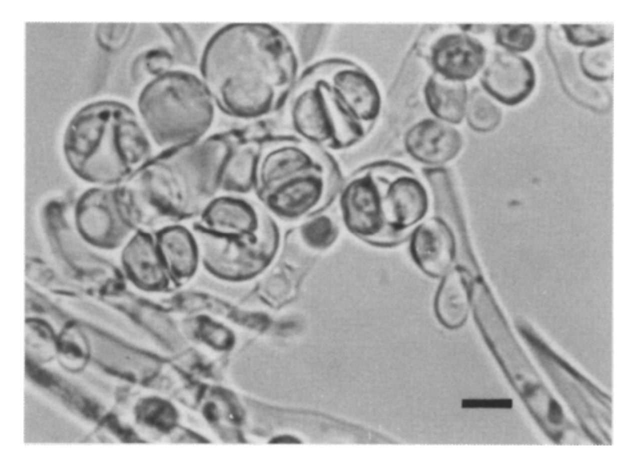


Fig. 165. *S. malanga*, CBS 6531. Asci with ascospores. Note angular cluster of 3-D-hydroxypalmitic acid crystals, lower right. After 3 days on YM agar at 25°C. Bar = 5 μm.

Fermentation:

Glucose	w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	w		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	+
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	+/w		

Co-Q: Not determined.
Mol% G + C: Not determined.

Origin of the strains studied: Ragi, Indonesia (2); Chinese yeast ball (chiu-yüeh), the commercial starter used for making lao-chao, a fermented rice product, Taiwan (7).

Type strain: CBS 6267 (NRRL Y-7175), the type strain of *Hansenula malanga*, isolated from ragi-tape, Indonesia, by Dwidjoseputro and Wolf (1970).

Comments: *S. malanga* was initially described as a species of *Hansenula* by Dwidjoseputro (Dwidjoseputro and Wolf 1970) because it was believed that this species assimilated nitrate as a sole source of nitrogen. *S. malanga* and *S. fibuligera* are common to starchy fermented foods, but the two species are readily separated by assimilation tests (Table 38) and from culture odor. *S. fibuligera* has a strong fruity odor whereas *S. malanga* has a faint acidic smell.

Cultures of *S. malanga* are usually characterized by the presence of crystals. Kurtzman et al. (1974) identified 3-D-hydroxypalmitic acid as a major component of these crystals.

46.7. *Saccharomycopsis schoenii* (Nadson & Krasil'nikov) Kurtzman & Robnett (1995)

Synonyms:
Endomyces schoenii Nadson & Krasil'nikov (1932)
Arthroascus schoenii (Nadson & Krasil'nikov) Bab'eva, Vustin, Naumov & Vinovarova (1985)
Pichia nonfermentans Nakase (1971b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are mostly elongated, (2.5–5.0) × (5.0–24.7) μm, and may be curved or with irregular constrictions. Some cells show short, hair-like extensions. Cells occur singly and in pairs. Budding is on a broad base. Growth is tannish-white and somewhat butyrous.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows moderately branched true hyphae without blastoconidia. Pseudohyphae are not present. Aerobic growth is tannish-white, faintly glistening with fine striations, somewhat butyrous, and oppressed except for a slightly raised center. Margins are finely lobed. A faint acidic odor is present.

Formation of ascospores: Ascus formation is preceded by conjugation between independent cells or between adjacent hyphal cells. Asci are usually spindle-shaped and deliquescent. One to four ascopores are formed in each ascus, and the spores are spheroidal or subspheroidal with a smooth surface and an equatorial or subequatorial ledge (Fig. 166).

Ascospores were observed on 5% malt extract, V8, YM and rice agars after 3–7 days at 25°C.

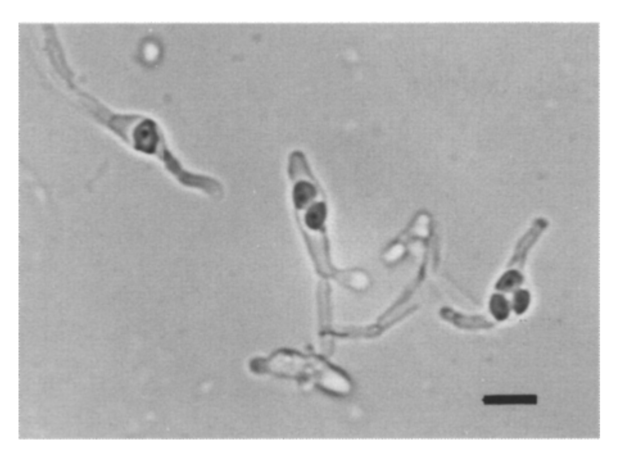


Fig. 166. *S. schoenii*, CBS 2556. Asci with ascospores. After 4 days on 5% malt extract at 25°C. Bar = 5 μm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	v	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8, CBS 7223 (Lee et al. 1994a).

Mol% G+C: 34.1, CBS 6423 (T_m: Nakase 1971b); 31.4–35.5, CBS 7223, 4 additional strains (T_m: Smith et al. 1990a).

Origin of the strains studied: CBS 7223 (VKM 1073, NRRL Y-17595) exudate of oak (*Quercus* sp.), de Hoog from VKM; CBS 2556 (NRRL Y-2389), slime flux of oak (*Quercus* sp.), Phaff; CBS 6423 (IFO 1579), soybean protein factory, Nakase, type strain of *P. nonfermentans*; CBS 6449, rotten tree trunk, van der Walt; CBS 7048, slime flux of oak (*Quercus* sp.), Bab'eva; CBS 7425, sour rot of grapes, Shann.

Type strain: CBS 7223.

Comments: In 1954, Kursanov noted the similarity of *Endomyces schoenii* with *Arthroascus javanensis*. Bab'eva et al. (1985) and Naumov et al. (1985b) concluded on the basis of genetic studies that *E. schoenii* should be assigned to *Arthroascus*, and that it is distinct from *A. javanensis*, thereby resulting in the reclassification of *E. schoenii* as *Arthroascus schoenii* (Bab'eva et al. 1985). The validity of this new combination was substantiated by Smith et al. (1990a) who showed low nDNA relatedness between *A. javanensis* and *A. schoenii*. In further comparisons, Smith et al. (1990a) showed high nDNA relatedness between *A. schoenii* and *Pichia nonfermentans*, a species earlier considered a synonym of *A. javanensis* by von Arx et al. (1977) on the basis of phenotypic similarity. Lee et al. (1994a) described a third species of *Arthroascus*, *A. fermentans*, and demonstrated that it had low nDNA relatedness with *A. javanensis* and *A. schoenii*. Kurtzman and Robnett (1994a, 1995) showed from rRNA/rDNA sequence comparisons that species of *Arthroascus* were closely related to members of *Saccharomycopsis* and proposed the transfer of *Arthroascus* species to *Saccharomycopsis*. This transfer is discussed further under Comments on the genus.

46.8. *Saccharomycopsis selenospora* (Nadson & Krasil'nikov) Kurtzman & Robnett (1995)

Synonyms:

Guilliermondella selenospora Nadson & Krasil'nikov (1928)

Endomycopsis selenospora (Nadson & Krasil'nikov) Dekker (Stelling-Dekker 1931)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal to long ellipsoidal, (2.0–5.0)×(5.0–15.0) μm, and occur singly or in pairs. Budding may be on either a narrow or a somewhat broad base. Thin, short cellular extensions often occur giving cells a knobby appearance. Growth is dull and roughened.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, moderately branched true hyphae with few blastoconidia. Pseudohyphae are infrequent. Aerobic growth is dull, white and mycelial with deep striations that have a segmented appearance. Margins are finely lobed with a narrow band of subaerial mycelium. An acidic, ester-like odor is present.

Formation of ascospores: Asci are somewhat spindle-shaped and either form at hyphal tips or they can be intercalary. Occasionally, asci form between adjacent hyphae giving the impression of having arisen following conjugation. Ascospores are spheroidal, ovoidal or reniform and those of some strains have terminal appendages that are extensions of the cell wall (Kreger-van Rij and van der Walt 1963) (Fig. 167). Four spores are usually formed in each ascus and asci deliquesce at maturity. Kreger-van Rij (1970b) reported that single-spore isolates are ascosporogenous indicating the species to be homothallic.

Ascospores were observed on YM agar after 4–7 days at 25°C.

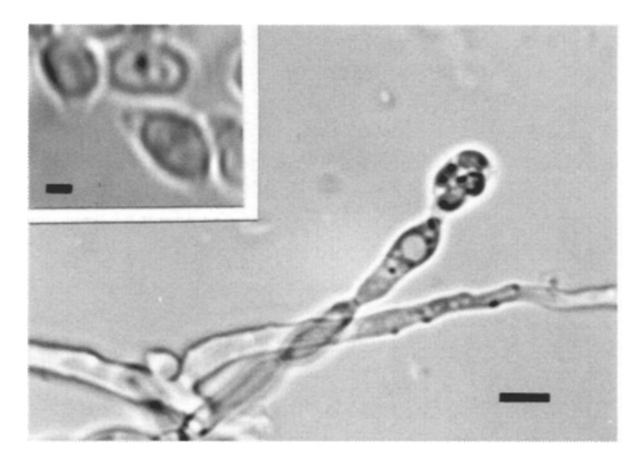


Fig. 167. *S. selenospora*, CBS 2563. An ascus with ascospores. After 10 days on YM agar at 25°C. Bar = 5 μm. Inset: Free ascospores showing terminal extensions of the spore wall. Bar = 1 μm.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	s
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8, CBS 2562 (Yamada et al. 1976b).
Mol% G + C: 30.2, S.A. Meyer (unpublished).

Origin of the strains studied: CBS 2562 (NRRL Y-1357) was originally from Guilliermond, but the source of isolation is unknown; CBS 2563 (NRRL Y-17724) was isolated from tanning fluid in France and originally supplied by J. Boidin.

Type strain: CBS 2562.

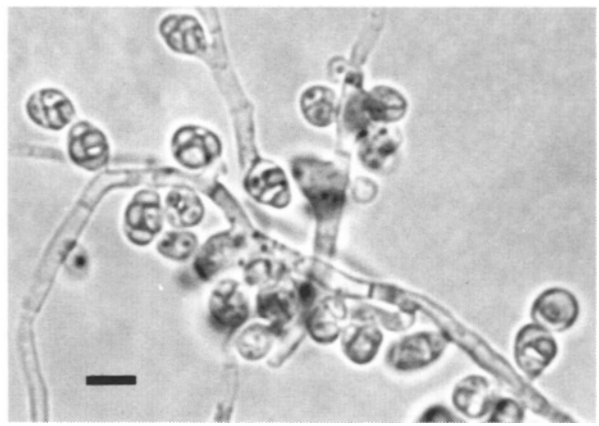


Fig. 168. *S. synnaedendra*, CBS 6161. Asci with ascospores. After 2 weeks on potato-dextrose agar at 25°C. Bar = 5 μm.

46.9. *Saccharomycopsis synnaedendra* D.B. Scott & van der Walt (van der Walt and D.B. Scott 1971d)

Synonyms:
Botryoascus synnaedendrus (D.B. Scott & van der Walt) von Arx (1972)
Pichia microspora Batra (1971)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are infrequently spheroidal, but usually ellipsoidal to elongate, (2.0–4.0) × (3.0–5.0) μm, often tapered, and occur singly, in pairs, or in small clusters. Growth is tannish-white and mycelial.

Growth on the surface of assimilation media: Some strains form pellicles.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant, well-branched true hyphae that are devoid of blastoconidia. Pseudohyphae are rare and often only a few cells in length. Aerobic growth is dull, tannish-white, convoluted and mycelial. Margins are finely lobed. A faint acidic odor is present.

Formation of ascospores: Asci may be free or form terminally on the tips of hyphae, either singly or in chains of two or three, or hyphal cells may develop into asci. Asci are unconjugated, but conjugations between hyphae as well as between yeast cells are often seen. Each ascus contains two to four hat-shaped ascospores that are liberated at maturity (Fig. 168). It is not known whether the species is homothallic or heterothallic, but germinating ascospores often conjugate with each other raising the possibility of heterothallism.

Ascospores were observed on 5% malt extract and potato-dextrose agars after 3–7 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	v	Succinate	w/+
L-Arabinose	–	Citrate	v
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8, CBS 6161 (Yamada et al. 1976b).
Mol% G+C: 42.0, (S.A. Meyer, unpublished data).

Origin of the strains studied: CBS 6161 (NRRL Y-7466) tunnel of *Platypus externedentatus*, South Africa, J.P. van der Walt; CBS 6393 (NRRL Y-7404), type strain of *Pichia microspora*, from *Crossotarsus wollastoni* in *Dipterocarpus indicus*, India, L.R. Batra.

Type strain: CBS 6161.

Comments: In contrast to the original description of *S. synnaedendra* by Scott and van der Walt (in van der Walt and Scott 1971d), von Arx (1972) reported the absence of budding yeast cells in cultures of this species. Because members of the genus *Saccharomycopsis* typically form budding cells, von Arx (1972) described the genus *Botryoascus* to accommodate *S. synnaedendra*. Upon examining cultures of *S. synnaedendra*, Kreger-van Rij (1984f) detected budding cells and rejected the genus *Botryoascus*. Kurtzman and Robnett (1994a, 1995) compared rRNA/rDNA sequences from all known species of hyphal yeasts and found *S. synnaedendra*, as well as the other *Saccharomycopsis* species accepted in the present treatment, to be members of a single clade. Consequently, these data show that *Botryoascus* is not phylogenetically separate from *Saccharomycopsis*.

46.10. *Saccharomycopsis vini* (Kreger-van Rij) van der Walt & D.B. Scott (1971d)

Synonyms:
Endomycopsis vini Kreger-van Rij (1964a)
Endomycopsella vini (Kreger-van Rij) von Arx (van der Walt and von Arx 1980)

Growth on 5% malt extract agar: After 3 days at 25°C, budding cells are ovoidal to elongate and often tapered. Budding cells measure (1.9–4.0)×(3.8–14.1) µm, and occur singly, in pairs, and in small clusters. Growth is dull, tannish-white, and butyrous to mycelial.

Growth on the surface of assimilation media: Pellicles are formed on the surfaces of some media.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows abundant moderately branched true hyphae with occasional blastoconidia. Pseudohyphae are infrequent. Aerobic growth is tannish-white and dull with a white, powdery periphery of hyphae. Margins are broadly lobate. A faint, fruity odor is present.

Formation of ascospores: Asci are spheroidal to ellipsoidal and are commonly borne on short sterigma on the tips and sides of hyphal cells. Conjugation often occurs between cells before ascus formation. Asci are persistent and contain one to four spheroidal spores that have an equatorial ledge as well as a subequatorial ledge (Fig. 169). Wart-like protuberances on spore surfaces can be seen by SEM (Kurtzman and Wickerham 1973). Single-ascospore isolates give ascosporogenous colonies indicating the species to be homothallic.

Ascospore formation was observed on YM, 5% malt extract, and yeast morphology agars after 5–7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	v
Sucrose	v	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+/w
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+/w	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	v		

Co-Q: 8, CBS 4110 (Yamada et al. 1976b).
Mol% G+C: Not determined.

Origin of the strains studied: CBS 4110 (NRRL Y-7126, NRRL Y-7290), CBS 4109 (NRRL Y-7127), grapes, Chile; CBS 4097 (NRRL Y-7289), CBS 4376

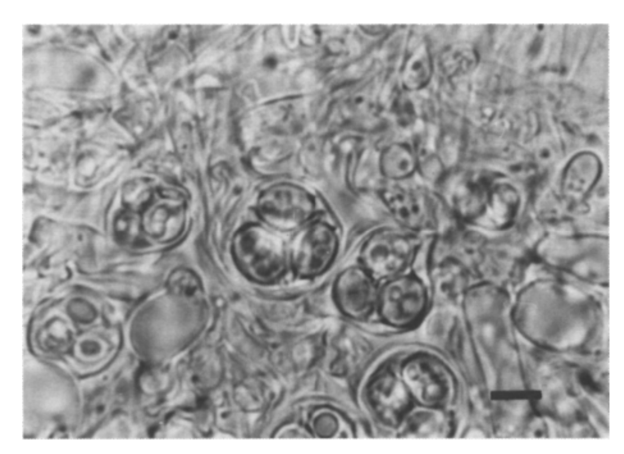


Fig. 169. *S. vini*, CBS 4097. Asci with ascospores. After 2 weeks on YM agar at 25°C. Bar = 5 μm.

(NRRL Y-7291), CBS 4377 (NRRL Y-7292), grape must, Brazil; NRRL Y-5905-Y-5909, NRRL Y-5911-Y-5916, hawthorne (*Crataegus* sp.) fruits, Peoria, Illinois, U.S.A.

Type strain: CBS 4110, from a grape, Chile.

Comments on the genus

The history of the genus *Saccharomycopsis* is perhaps the most convoluted of any of the ascomycetous yeast genera. Many of the species currently accepted in *Saccharomycopsis* were originally assigned to *Endomyces* Reess, a genus characterized by formation of true hyphae. Yeast phases of some of the species result from budding (blastoconidia), whereas those of the other species are from arthroconidium formation (fission). Stelling-Dekker (1931) used this morphological distinction to subdivide *Endomyces* and placed the budding species in the newly described genus *Endomycopsis* Dekker while reserving *Endomyces* for those species that form only arthroconidia. A number of other mycelial species, such as *Pichia bispora, P. burtonii, Ambrosiozyma monospora* and *A. platypodis,* were later assigned to *Endomycopsis* by various authors.

In 1971, van der Walt and Scott (1971d) pointed out that *Endomycopsis* Dekker was illegitimate and reinstated *Saccharomycopsis* Schiönning in its original context (i.e., presence of budding cells, true septate mycelium, and ascospores with double membranes) for placement of certain of the *Endomycopsis* species. Shortly thereafter, van der Walt (1972) described the genus *Ambrosiozyma* for mycelial species with dolipore-like septa. Von Arx (1972) promptly emended species assignments in the genus by describing *Hormoascus* for nitrate-assimilating species of *Ambrosiozyma.* Later, van der Walt and von Arx (1985) emended *Hormoascus* to include both nitrate-assimilating and non-assimilating species. *Botryoascus* was described for *Saccharomycopsis synnaedendra,* a species von Arx (1972) believed not to produce budding yeast cells, and *Arthroascus* was described for *Endomycopsis* (*Endomyces*) *javanensis* which forms spheroidal ascospores and buds on a broad base (von Arx 1972). Later, van der Walt and von Arx (1980) transferred *Saccharomycopsis lipolytica* to the genus *Yarrowia,* and *S. vini* and *S. crataegensis* to *Endomycopsella,* a genus known only from the illustrations of Boedijn (1960). *Saccharomycopsis fibuligera* was returned to *Endomyces* in a paper aptly titled "The adventures of the yeast genus *Endomycopsis* Dekker" (von Arx and Yarrow 1984).

The delimitation of *Endomyces* from *Dipodascus* and from *Saccharomycopsis* and its synonyms has been controversial for a number of reasons. The phylogenetic significance of blastoconidium and arthroconidium formation has been interpreted many ways, as has the significance of ascospore morphology and the ultrastructure of hyphal septa. For example, von Arx and van der Walt (1987) aligned genera of the Endomycetales primarily on the basis of ascospore shape and their possible relatedness to members of the euascomycetes. Additionally, the characteristics of *Endomyces decipiens,* the type species of the Order Endomycetales, are known only from the drawings of Brefeld (von Arx 1972, von Arx and Yarrow 1984) and subject to wide interpretation. In an effort to preserve the nomenclatural stability of the Endomycetales, Redhead and Malloch (1977) designated as neotype a specimen identified as *Endomyces decipiens* that was found on *Agaricus melleus.*

Kurtzman and Robnett (1994a, 1995) approached the taxonomic dilemma of the Endomycetales from a molecular phylogenetic perspective. Partial sequences of rRNA/rDNA were compared from the type species of all cultivatable yeast genera as well as from all known species of the mycelial yeasts. These data demonstrated that the yeasts and yeastlike taxa form a clade separate from *Schizosaccharomyces, Taphrina/Protomyces,* and the euascomycetes. The rDNA analyses also showed that a strain identified as *Endomyces decipiens* aligned with the yeast clade, but a strain identified as *Endomyces scopularum* proved to be a euascomycete, thus raising doubts about the phylogenetic affinities of the Order Endomycetales and providing a reason for use of the Order Saccharomycetales for those taxa traditionally assigned to the Endomycetales.

The preceding phylogenetic analysis demonstrated that species accepted in the present treatment of *Saccharomycopsis* form a distinct clade that is separate from other mycelial genera (Fig. 170) as well as from such species as *Pichia burtonii, P. scolyti, P. bispora* and *P. wickerhamii* (C.P. Kurtzman and C.J. Robnett, manuscript in preparation). Members of *Saccharomycopsis* show considerable species-specific variation in the shape of their ascospores, which may be hat-shaped (galeate) or spheroidal to elongate, and with or without equatorial ledges or short polar appendages. Asci are generally spheroidal to ellipsoidal and either free or attached to hyphae. Species previously assigned to *Arthroascus* and *Guilliermondella* are morphologically distinguished from one another by ascospore shape and from the remainder of the species in *Saccharomycopsis* by the appearance of their asci, which are often transformed hyphal cells

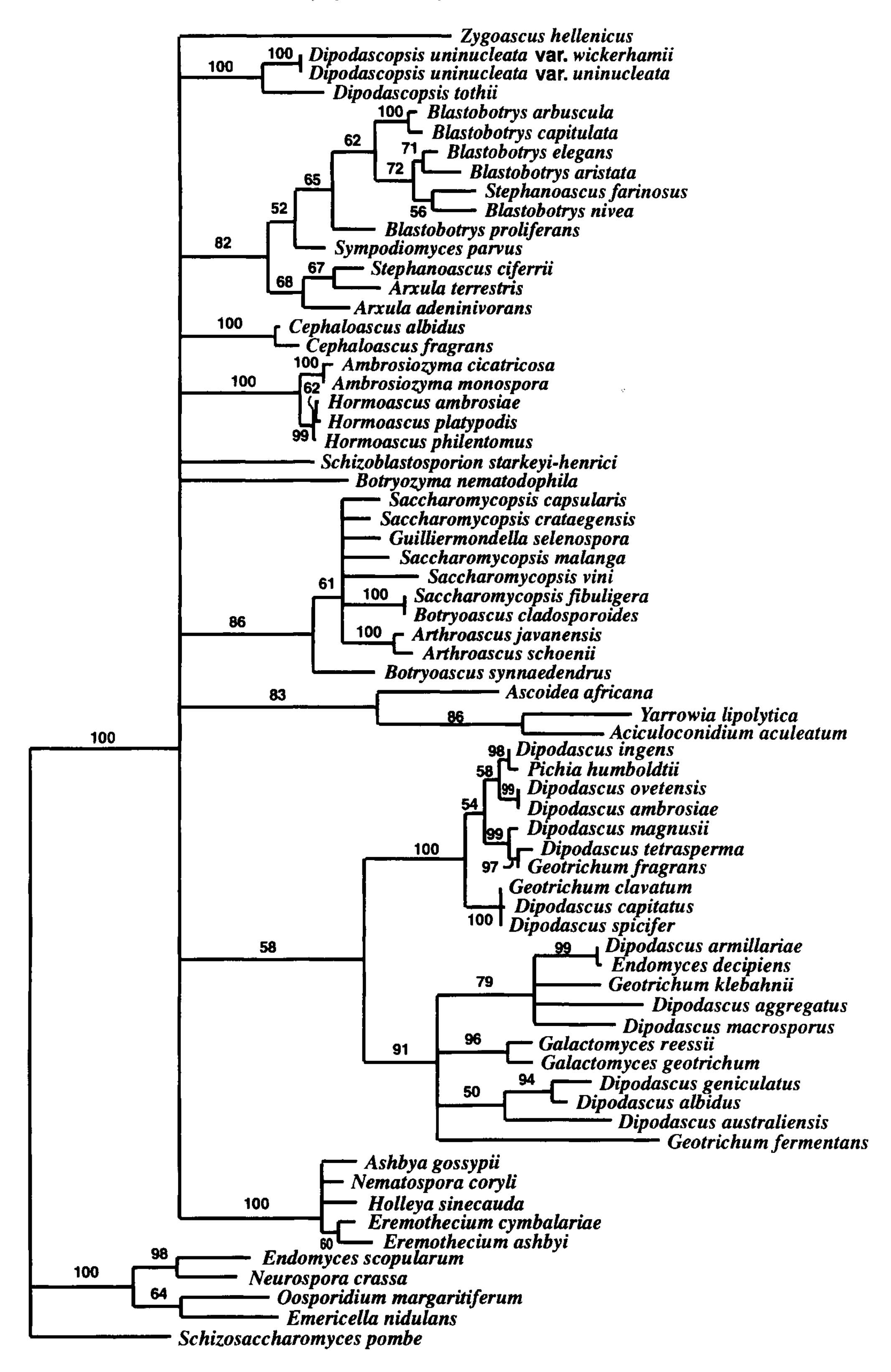


Fig. 170. A phylogenetic tree derived from maximum parsimony analysis depicting the mycelial ascomycetous yeasts, yeastlike fungi, and various reference species (Kurtzman and Robnett 1995). The phylogram was calculated from nucleotide divergence in the 5′ end of the large subunit rRNA gene (positions 63–680). Branch lengths are proportional to nucleotide differences, and the numbers given on branches are the percentage of frequencies with which a given branch appeared in 100 bootstrap replications. Branches with frequencies of less than 50% were collapsed.

that become spindle-shaped as the ascospores are formed (Kreger-van Rij 1984c,e). Hyphal septa of all species assigned to *Saccharomycopsis* are multiperforate with the exception of *S. javanensis,* which has septa with a single central micropore. Consequently, phylogenetic analysis of rDNA nucleotide sequences shows that ascospore shape, and to a lesser extent, septum ultrastructure, are not genus-specific characters.

47. *Saturnispora* Liu & Kurtzman[1]

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or somewhat elongate. True hyphae are not formed but pseudohyphae may occur. Certain of the species form pellicles on the surface of liquid media.

Asci may be unconjugated or show conjugation between a parent cell and its bud or between independent cells. Asci may be either persistent or deliquescent and form 1–4 or, rarely, 5–8 spheroidal ascospores which are ornamented with an equatorial ledge.

Glucose is fermented. Neither nitrate nor D-xylose is assimilated. Diazonium blue B reaction is negative.

Type species

Saturnispora dispora (Dekker) Liu & Kurtzman

Species accepted

1. *Saturnispora ahearnii* Kurtzman (1991)
2. *Saturnispora dispora* (Dekker) Liu & Kurtzman (1991)
3. *Saturnispora saitoi* (K. Kodama, Kyono & S. Kodama) Liu & Kurtzman (1991)
4. *Saturnispora zaruensis* (Nakase & Komagata) Liu & Kurtzman (1991)

Key to species

See Table 39.

1. a Trehalose assimilated → 2
   b Trehalose not assimilated .... *S. ahearnii*: p. 387

2(1). a Pellicles formed on the surface of liquid growth media .... *S. saitoi*: p. 389
   b Pellicles not formed on the surface of liquid growth media .... *S. dispora*: p. 388
   S. zaruensis: p. 390

Table 39
Key characters of species in the genus *Saturnispora*

Species	Assimilation		Pellicle[a]	Deliquescent asci	Number of spores per ascus	Circumfluent ledge on ascospores
	Trehalose	Ribitol				
Saturnispora ahearnii	–	–	+	+	1–4	+
S. dispora	+	+	–	–	1–2	–
S. saitoi	+	v	+	+	1–4	+
S. zaruensis	+	+	–	–	1–2	+

[a] Formation of a pellicle on the surface of liquid growth media.

Systematic discussion of the species

47.1. *Saturnispora ahearnii* Kurtzman (1991b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal or somewhat elongate, (4.0–7.0)×(4.2–12.0) µm, and occur singly or occasionally in pairs.

Growth on the surface of assimilation media: Thin, climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, colonies are low convex, tannish-white in color with a smooth to faintly striated, semi-glistening surface and a butyrous texture. Colony margins are smooth to slightly scalloped. Hyphae or pseudohyphae are not formed.

Formation of ascospores: Asci are either unconjugated or show conjugation between a cell and its bud. The asci form 1–4 or, rarely, 5–8 spheroidal ascospores that have a thin equatorial ledge. Asci are deliquescent and release their spores soon after formation. Isolation of single ascospores demonstrated this species to be homothallic (Kurtzman 1991b).

Ascospores form abundantly within 2–3 days at 25°C on 5% malt extract and YM agars.

[1] The original spelling of the genus name *Saturnospora* has been treated as an orthographic error.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 34.9, CBS 6121 (BD: Kurtzman 1991a).

Origin of the strain studied: CBS 6121 (NRRL Y-7555) is the only known strain of *S. ahearnii* and was isolated from muddy water in the rhizosphere of oyster grass (*Spartina alterniflora* Loisel) growing in the salt marshes of Barataria Bay in southeastern Louisiana, U.S.A.

Type strain: CBS 6121 (NRRL Y-7555), received from D.G. Ahearn, U.S.A.

Comments: *S. ahearnii* is an interesting species because of its close relatedness to *S. saitoi*. *S. ahearnii* initially appeared to be a strain of *S. saitoi* that failed to assimilate trehalose. Comparisons of nuclear DNA complementarity showed the two taxa to have 30% relatedness (Kurtzman 1991b), thus placing them in the category of genetic sibling species that have only recently diverged (Kurtzman 1987b). All known strains of *S. saitoi* are from Japan, but speculation on whether or not the genetic separation of these two species can be attributed to allopathy is premature until additional strains of both species are isolated and there is a better understanding of their geographical ranges.

47.2. *Saturnispora dispora* (Dekker) Liu & Kurtzman (1991)

Synonyms:
Saccharomyces disporus Beijerinck (1908) nom. nud.
Debaryomyces disporus Dekker (Stelling-Dekker 1931)
Zymodebaryomyces disporus (Dekker) Novák & Zsolt (1961)
Pichia dispora (Dekker) Kreger-van Rij (1970c)
?*Pichia krusei* Tsuchiya, Fukazawa, Shinoda & Imai (1967) nom. nud.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.5–5.5)×(2.5–6.5) µm, and occur singly and in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Rings of growth may occur, but pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither hyphae nor pseudohyphae but only an occasional outgrowth of elongated cells. Aerobic growth is tannish-white, butyrous, smooth, glistening, and with an occasionally lobed margin.

Formation of ascospores: Asci may be unconjugated or, more frequently, they result from conjugation between a parent cell and a bud or from the pairing of independent cells. Each ascus contains one or two spheroidal spores that exhibit an incomplete equatorial ledge which may be barely perceptible under the light microscope (Fig. 171). Asci are persistent.

Ascospores were observed on 5% malt extract and YM agars after 10–20 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	w/–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7 (Yamada et al. 1973a).
Mol% G+C: 37.6, CBS 794 (T_m: Nakase 1972a); 38.0, CBS 794, NRRL Y-7656 (BD: Kurtzman 1991a).

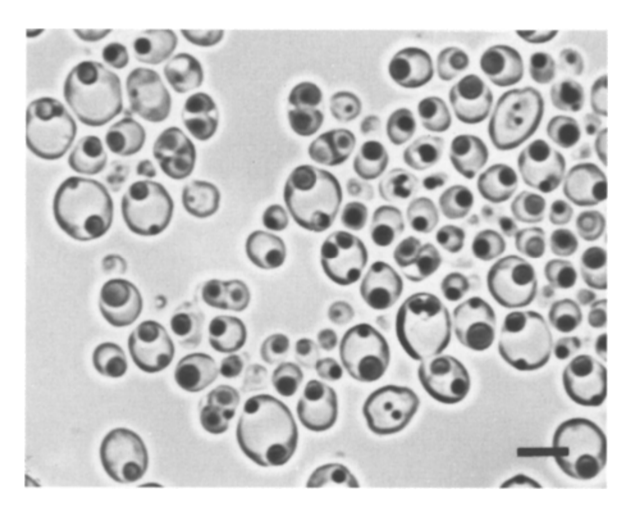


Fig. 171. *S. dispora*, CBS 794. Ascus with ascospores, after 15 days on YM agar, 25°C. Bar = 5 µm.

Origin of the strains studied: CBS 794 (NRRL Y-1447), Beijerinck's strain from the exudate of an oak (*Quercus* sp.); NRRL Y-7656 (LKB 801), a strain of unknown origin from K. Kodama, Japan.

Type strain: CBS 794 (NRRL Y-1447), Beijerinck's strain.

Comments: Kreger-van Rij (1970c) transferred this species from *Saccharomyces* to *Pichia* because of the presence of a ledge on the ascospores, but noted that in the transmission electron microscope, the ledge appeared not to be circumfluent. Using the scanning electron microscope, Kurtzman and Smiley (1974) confirmed the thin ledge to traverse only about half of the ascospore circumference. This is in contrast to the other species in the genus which have ascospores with circumfluent ledges. The taxon described as *P. krusei* is considered to be a synonym of *S. dispora*, but this needs to be verified from comparisons of DNA relatedness. Separation of *S. dispora* and *S. zaruensis* is discussed under the latter species.

47.3. *Saturnispora saitoi* (K. Kodama, Kyono & S. Kodama) Liu & Kurtzman (1991)

Synonym:
Pichia saitoi K. Kodama, Kyono & S. Kodama (1962)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are ovoidal, (2.3–4.5)×(2.8–7.5) µm, and occur singly and in pairs. Growth is butyrous and tannish-yellow in color.

Growth on the surface of assimilation media: Climbing pellicles are present.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass may show pseudohyphae but not true hyphae. Aerobic growth is light tan, smooth, glistening, butyrous and with a lobate margin.

Formation of ascospores: Asci are generally unconjugated but occasional conjugations occur between a cell and its bud. Two to four saturn-shaped spores are produced in each ascus, but the ledge on the spores is sometimes difficult to discern under the light microscope (Fig. 172). The spores are released soon after formation. This species appears to be homothallic. Single-spore isolates from four-spored asci of the type strain gave approximately two-thirds sporogenous colonies and one-third asporogenous colonies. Mixtures of asporogenous cultures showed neither conjugations nor spores. Single-spore isolations from three additional four-spored strains gave only sporogenous cultures (Kurtzman, unpublished data).

Ascospores were observed on YM- and 5% malt extract agars after 2–7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

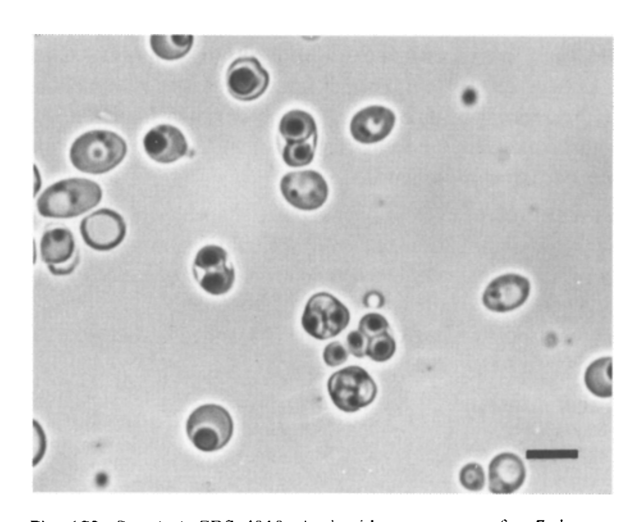


Fig. 172. *S. saitoi*, CBS 4910. Asci with ascospores, after 7 days on YM agar, 25°C. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	+
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7 (Yamada et al. 1973a).

Mol% G+C: 32.2–33.4, four strains (T_m: Nakase 1972a); 33.0–34.1, CBS 4910, NRRL Y-7820, NRRL Y-7821 (BD: Kurtzman 1991a).

Origin of the strains studied: CBS 4910 (NRRL Y-6671), from exudate of hornbeam (*Carpinus* sp.); NRRL Y-7655, NRRL Y-7820 through NRRL Y-7826, from K. Kodama, Japan.

Type strain: CBS 4910 (NRRL Y-6671), from exudate of *Carpinus* sp.

Comments: The close relatedness between *S. saitoi* and *S. ahearnii* is discussed under the Comments section of the latter species. There are few phenotypic differences upon which to separate species of *Saturnispora.* Nakase (1972a) reported that some strains of *S. saitoi* assimilate ribitol, thus precluding separation of this species from *S. dispora* and *S. zaruensis* by growth differences on standard carbon assimilation tests. However, strains of *S. saitoi* form pellicles on liquid media and have deliquescent asci, characters not shared by isolates of *S. dispora* and *S. zaruensis.*

47.4. *Saturnispora zaruensis* (Nakase & Komagata) Liu & Kurtzman (1991)

Synonyms:

Pichia zaruensis Nakase & Komagata (1966)

Candida agrestis S. Goto & Oguri (1983)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.3–5.6)×(2.5–6.6) µm, and occur singly and in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed, but thin rings of surface growth may occur.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither hyphae nor pseudohyphae. Aerobic growth is tannish-white, butyrous, smooth, glistening, and with margins that are occasionally lobed.

Formation of ascospores: Asci may be unconjugated or show conjugation between a parent cell and a bud or between independent cells. Asci contain one or usually two saturnoid spores. Asci are persistent.

Ascospores were formed on YM and 5% malt extract agars after 10–15 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7 (Yamada et al. 1973a).

Mol% G+C: 39.5, 39.6, CBS 5799, NRRL Y-7009 (BD: Kurtzman 1991a).

Origin of the strains studied: CBS 5799 (NRRL Y-7008) from exudate of an oak (*Quercus serrata* complex), Japan; NRRL Y-7009 (AJ 4143) from soil, Japan.

Type strain: CBS 5799 (NRRL Y-7008), from exudate of *Quercus serrata.*

Comments: Because there appear to be no differences in growth reactions or cellular morphology, *S. zaruensis* has been considered a synonym of *S. dispora* (Kreger-van Rij 1970c, Kurtzman 1984d). However, comparisons of nuclear DNA relatedness revealed the two taxa to be separate species (Kurtzman 1991a). Kurtzman (1991a) examined the banding patterns generated by SDS-PAGE electrophoresis of unpurified cellular proteins from all four *Saturnispora* species and noted that this relatively rapid method allowed separation of *S. dispora* and *S. zaruensis.* Additionally, as seen under the scanning electron microscope, ascospores of *S. zaruensis* have a heavy circumfluent ledge, whereas the equatorial ledge on ascospores of *S. dispora* is incomplete (Kurtzman and Smiley 1974, unpublished data).

Comments on the genus

The genus *Saturnispora* was described by Liu and Kurtzman (1991) for certain saturn-spored species that had been maintained in *Pichia* (Kreger-van Rij 1970c, Kurtzman 1984d). The relationships among the saturn-spored yeasts are discussed in the chapter on the genus *Williopsis.*

48. *Schizosaccharomyces* Lindner

Ann Vaughan-Martini and Alessandro Martini

Diagnosis of the genus

Cells are globose to cylindroidal and reproduce by fission. In some species true septate hyphae develop and break into arthrospores.

Asci are usually produced by somatic conjugation of vegetative cells. Ascospores may be liberated at an early stage. Ascospores are globose or short ellipsoidal, seldom cylindroidal.

Sugars are fermented. Nitrate is not assimilated. Positive starch test in Wickerham's liquid medium. Urea is hydrolyzed. Diazonium blue B reaction is negative.

Type species

Schizosaccharomyces pombe Lindner

Species accepted

1. *Schizosaccharomyces japonicus* Yukawa & Maki (1931)
2. *Schizosaccharomyces octosporus* Beijerinck (1894)
3. *Schizosaccharomyces pombe* Lindner (1893)

Key to species

See Table 40.

1. a Usually four spores per ascus, D-gluconate assimilated .... *S. pombe*: p. 393
   b Usually six to eight spores per ascus, D-gluconate not assimilated → 2

2(1). a Raffinose and sucrose fermented, formation of true septate hyphae .... *S. japonicus*: p. 391
   b Raffinose and sucrose not fermented, true septate hyphae not present .... *S. octosporus*: p. 392

Table 40
Key characters of species of the genus *Schizosaccharomyces*

Species	Fermentation		Assimilation			Growth at		Number of ascospores	True hyphae	Co-Q[a]
	Sucrose	Raffinose	Sucrose	Raffinose	D-Gluconate	32°C	37°C			
Schizosaccharomyces japonicus	+	+	+	+	–	+	+	6–8	+	n.d.
S. octosporus	–	–	–	–	–	–	–	6–8	–	Q-9
S. pombe	+	+	+	+	+	v	–	4	–	Q-10

[a] Co-Q data from Yamada et al. (1973b); n.d.: not detected.

Systematic discussion of the species

48.1. *Schizosaccharomyces japonicus* Yukawa & Maki (1931)

Synonyms:

Schizosaccharomyces versatilis Wickerham & Duprat (1945) nom. nud.

Schizosaccharomyces japonicus Yukawa & Maki var. *versatilis* (Wickerham & Duprat) Slooff (1970)

Octosporomyces japonicus (Yukawa & Maki) Kudryavtsev (1960)

Hasegawaea japonica (Yukawa & Maki) Y. Yamada & Banno (1987a)

Hasegawaea japonica (Yukawa & Maki) Y. Yamada & Banno var. *versatilis* (Wickerham & Duprat) Y. Yamada & Banno (1987b)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, subglobose, ellipsoidal or occasionally cylindroidal, (5.0–9.0)×(6.0–22.0) μm, single, in pairs or in small groups. Ascospores may be present. A sediment is present at 3 days and after one month at 20°C as well.

Growth on 5% malt agar: After one month at 20°C, the streak culture is grayish to light brown, glistening, and slightly raised in the center with irregular striations; the margin is fringed with hyphae.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 5 days at 25°C, true septate hyphae and arthrospores are formed. Conjugating cells as well as ascospores may be present.

Formation of ascospores: Conjugation of cells usually precedes the formation of asci which contain six to eight, sometimes fewer, globose to ellipsoidal ascospores. The asci are evanescent, and released ascospores may remain together in chains or clumps. Mature ascospores of some

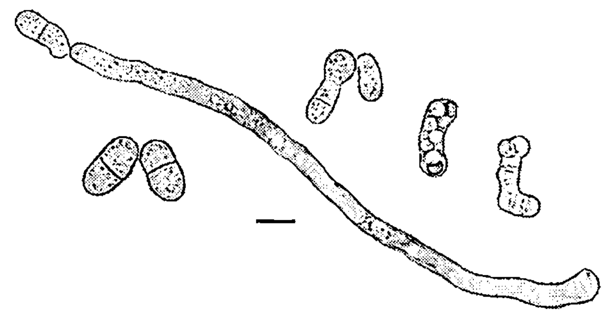


Fig. 173. *S. japonicus*, CBS 354. True mycelium, vegetative and conjugating cells and 8-spored asci after 3 days. Potato agar, 25°C. Bar = 10 µm.

strains may be reniform to allantoid and give an amyloid reaction with Lugol's iodine. Conjugation may also occur between ascospores (Fig. 173). When observed by SEM, the ascospores present a smooth surface (Mikata and Banno 1987).

Sporulation was observed on malt-, potato- and corn meal agars after 7 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	v	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	v	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm cycloheximide	–
L-Lysine	–	Growth at 37°C	+
Ethylamine·HCl	–		

Co-Q: not detected (Yamada et al. 1973b).
Mol% G+C: 32.3–36.3, CBS 103, CBS 354, DBVPG 6573 (T_m: Vaughan-Martini, unpublished data)
Linoleic acid: present (Kock and van der Walt 1986).

Origin of the strains studied: DBVPG 6274 (CBS 354) from strawberry wine; DBVPG 6351 (CBS 103), type strain of *S. japonicus* var. *versatilis*; DBVPG 6371 (CBS 5679) from tree exudate; DBVPG 6416 (CBS 102) from grape juice.

Type strain: CBS 354 (DBVPG 6274), isolated from strawberry wine by Yukawa and Maki (1931).

Comments: The high DNA relatedness between strains representing the two varieties of *S. japonicus* demonstrated that there is little reason to separate the species into the varieties *japonicus* and *versatilis* (Vaughan-Martini 1991). This separation was originally based upon a slight difference in the shape and iodine reaction of mature ascospores from some strains (Slooff 1970b, Yarrow 1984b). Johannsen (1981) and Sipiczki et al. (1982) found high percentages of mating between strains of *S. japonicus* var. *japonicus* and *S. japonicus* var. *versatilis,* indicating conspecificity. A study of electrophoretic mobility of selected enzymes of four strains representing the two varieties showed identical profiles (Yamada et al. 1987a).

48.2. *Schizosaccharomyces octosporus* Beijerinck (1894)

Synonyms:
Octosporomyces octosporus (Beijerinck) Kudryavtsev (1960)
Schizosaccharomyces slooffiae Kumbhojkar (1972)

Growth in 5% malt extract: After 3 days at 25°C, the cells are subglobose, (4.4–10.0) µm, to cylindroidal, (4.5–7.0) × (9.0–22.0) µm, single, in pairs or small groups. A sediment is present at 3 days and after one month at 20°C as well.

Growth on 5% malt agar: After one month at 20°C, the streak culture is gray to grayish-brown, dull, raised irregularly and striated; the margin is entire, sinuous or lobate.

Growth on the surface of assimilation media: Pellicles are not formed.

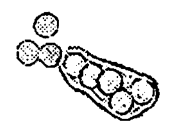
Dalmau plate culture on morphology agar: After 5 days at 25°C, hyphae were not observed. Ascospores may be present.

Formation of ascospores: Conjugation of cells usually precedes the formation of asci containing six to eight, or fewer, ascospores that are globose to short ellipsoidal. Conjugation may also occur between ascospores (Fig. 174). When observed by SEM ascospore surfaces appear papillate (Mikata and Banno 1987).

Sporulation was observed on malt-, potato- and corn meal agars after 7–9 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	v	Melibiose	–



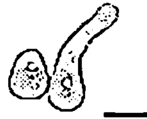



Fig. 174. *S. octosporus*, CBS 371. Conjugating cells, 8-spored ascus and asci releasing ascospores, after 5 days, malt agar, 25°C. Bar = 10 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm cycloheximide	–
L-Lysine	–	Growth at 37°C	–
Ethylamine·HCl	–		

Co-Q: 9 (Yamada et al. 1973b).
Mol% G+C: 40, 1 strain (BD: Storck 1966); 38.9, CBS 371 (T_m: Vaughan-Martini, unpublished data).
Linoleic acid: not present (Kock and van der Walt 1986).

Origin of the strains studied: DBVPG 4091 and DBVPG 4092 from honey; DBVPG 4093 and DBVPG 4094 from dried figs; DBVPG 6276 (CBS 371) from currants; DBVPG 6278 (CBS 6207) from honey; DBVPG 6434 (CBS 2632) unknown; DBVPG 6443 (CBS 6209), DBVPG 6471 (CBS 373) and DBVPG 6472 (CBS 6206) from honey.

Type strain: CBS 371 (ATCC 4206, DBVPG 6276, NRRL Y-855), isolated from Greek currants by Beijerinck, and chosen as the type by Lodder and Kreger-van Rij (1952).

48.3. *Schizosaccharomyces pombe* Lindner (1893)

Synonyms:
Schizosaccharomyces asporus Beijerinck (1897)
Saccharomyces pombe (Lindner) Jörgensen (1898)
Schizosaccharomyces vordermanii Wehmer (1906)
Schizosaccharomyces mellacei Jörgensen (1909)
Schizosaccharomyces formosensis Nakazawa (1914)
Schizosaccharomyces formosensis Nakazawa var. *akoensis* Nakazawa (1914)
Schizosaccharomyces formosensis Nakazawa var. *tapaniensis* Nakazawa (1914)
Schizosaccharomyces santawensis Nakazawa (1914)
Schizosaccharomyces pinan Nakazawa (1919)
Schizosaccharomyces taito Nakazawa (1919)
Schizosaccharomyces liquefaciens Osterwalder (1924b)
Schizosaccharomyces acidodevoratus Tschalenko (1941)
Schizosaccharomyces malidevorans Rankine & Fornachon (1964)
Schizosaccharomyces pombe Lindner var. *malidevorans* (Rankine & Fornachon) Sipiczki, Kucsera, Ulaszewski & Zsolt (1982)
Schizosaccharomyces pombe Lindner var. *acidodevoratus* (Tschalenko) Dittrich (1964)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose, ellipsoidal or cylindroidal, (3.0–5.0)×(5.0–15.0–24.0) μm, single, in pairs or small groups. After one month at 20°C, a sediment is present and sometimes a thin ring.

Growth on 5% malt agar: After one month at 20°C, the streak culture is brownish, dull or glistening, slightly raised, striated, and the margin is entire or sinuous.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 5 days at 25°C, a few short chains of cells may be observed. Ascospores may be present.

Formation of ascospores: Conjugation of vegetative cells precedes the formation of evanescent asci containing two to four globose to ellipsoidal ascospores. The ascospores may cohere in small groups upon release (Figs. 175, 176). Haploid heterothallic strains may be encountered. When observed by SEM, the ascospore surface is warty (Mikata and Banno 1987).

Sporulation was observed on malt-, potato- and corn meal agars after 5–6 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	v
Sucrose	+	Trehalose	–
Maltose	+	Melibiose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Cadaverine·HCl	–	100 ppm cycloheximide	–
L-Lysine	–	Growth at 37°C	–
Ethylamine·HCl	–		

Co-Q: 10 (Yamada et al. 1973b).
Mol% G+C: 42, 1 strain (T_m: Rost and Venner 1964); 35.1–37.5, CBS 356, CBS 357, CBS 1042, CBS 1061, CBS 5557 (T_m: Vaughan-Martini, unpublished data).
Linoleic acid: not present (Kock and van der Walt 1986).

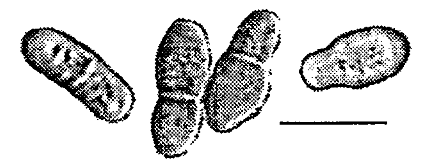


Fig. 175. *S. pombe*, CBS 356. Vegetative cells after 2 days on YEPG agar, 25°C. Bar = 10 μm.

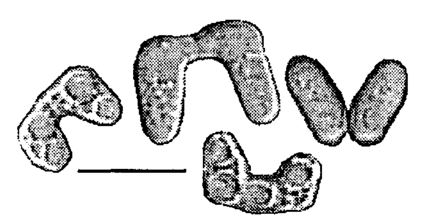


Fig. 176. *S. pombe*, CBS 5557. Conjugating cells and 4-spored asci after 6 days on YEPG agar, 25°C. Bar = 10 μm.

Origin of the strains studied: DBVPG 6277 (CBS 356) from arak mash; DBVPG 6280 (CBS 357) from cane sugar molasses; DBVPG 6373 (CBS 352) from arak mash; DBVPG 6374 (CBS 358); DBVPG 6418 (CBS 374) from molasses; DBVPG 6448 (CBS 5680) from apples; DBVPG 6450 (CBS 1063) from cane sugar molasses.

Complementary mating types: CBS 358 and CBS 1057.

Type strain: CBS 356 (DBVPG 6277), received from the Král collection in 1922, and designated as the type by Lodder and Kreger-van Rij (1952).

Comments: The distinction between *S. pombe* and *S. malidevorans* was not justified (Vaughan-Martini 1991) after DNA reassociation studies revealed that the type strains share 98% of their genomes. Conspecificity was proposed by Sipiczki et al. (1982) who found mating in all crosses between strains of the two taxa, as well as recombination between their mitochondrial genomes, and similar respiratory chains, as revealed by low temperature cytochrome spectra. Identical electrophoretic enzyme mobilities were found by Yamada et al. (1987a) for all strains of the group.

Comments on the genus

Low relatedness as seen from DNA reassociation studies confirmed the separation of the genus *Schizosaccharomyces* into three species: *S. japonicus*, *S. octosporus*, and *S. pombe* (Vaughan-Martini 1991). This was also demonstrated from hybridization studies (Johannsen 1981, Sipiczki et al. 1982) where interspecific mating was consistently absent.

Although a close relationship between 8- and 4-spored species was postulated by Kossikov and Medvedeva (1977), who reported mating between *S. pombe* and *S. japonicus,* complete documentation is lacking as to the identity and stability of this hybrid. Sipiczki (1979) also obtained a limited number of prototrophic hybrids by protoplast fusion between recognized strains of the species *S. pombe* and *S. octosporus*. It has been postulated, however, that fusion products do not necessarily reflect conspecificity, but rather are the result of a limited genetic exchange still to be clearly defined (Spencer et al. 1985, Vaughan-Martini 1991). In contrast, Sipiczki et al. (1982) found that *S. japonicus* and related strains had similar low-temperature cytochrome spectra, which were quite different from those of *S. pombe* and *S. octosporus*.

Over the years, different authors have called for the separation of *Schizosaccharomyces* into two or even three genera based on studies using various techniques such as composition of coenzyme Q (Yamada et al. 1973b), low-temperature cytochrome spectra (Sipiczki et al. 1982), fatty acid profiles (Kock and van der Walt 1986), and ascospore surface morphology as seen by SEM (Mikata and Banno 1987). As a result of these studies, Yamada and Banno (1987a) proposed the creation of the genus *Hasegawaea* for the species *S. japonicus,* the reinstatement of the genus *Octosporomyces* for the species *S. octosporus,* and the maintenance of *Schizosaccharomyces* for *S. pombe.*

Kurtzman & Robnett (1991), in a study of ribosomal RNA, found that strains comprising *S. japonicus* were somewhat divergent from the other two species of the genus. However, these authors felt that the distances were not sufficiently great to justify the division of the genus *Schizosaccharomyces* as proposed by Yamada and Banno (1987a).

49. *Sporopachydermia* Rodrigues de Miranda

M.A. Lachance and H.J. Phaff

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are ovoid, ellipsoidal or elongate, occasionally curved. Pseudomycelium and true mycelium are not formed. Cultures growing on agar media have a strong, unpleasant odor.

Conjugation may or may not precede ascus formation. The ascospores are spheroidal, subglobose, ellipsoidal, or bacilliform. One to four ascospores are formed per ascus. The spores usually have a thick coat of refractile material. They are liberated from the ascus at maturity, and are kept together by the refractile material.

Glucose fermentation is absent or very weak. Nitrate is not assimilated. Inositol as sole carbon source is assimilated. Coenzyme Q-9 is formed. Diazonium blue B reaction can be negative or positive.

Type species

Sporopachydermia lactativora Rodrigues de Miranda

Species accepted

1. *Sporopachydermia cereana* Rodrigues de Miranda (1978)
2. *Sporopachydermia lactativora* Rodrigues de Miranda (1978)
3. *Sporopachydermia quercuum* Lachance (1982)

Key to species

See Table 41.

1. a Erythritol assimilated .... *S. cereana*: p. 395
   b Erythritol not assimilated → 2

2(1). a Growth at 37°C .... *S. lactativora*: p. 396
   b Growth absent at 37°C .... *S. quercuum*: p. 397

Table 41
Key characters of species in the genus *Sporopachydermia*

Species	Glucose fermentation	Assimilation							Growth at 37°C	Curved cells
		Erythritol	Ribitol	Salicin	Succinate	Acetone	Tannic acid	Cadaverine[a]		
Sporopachydermia cereana	–	+	+	+	w/–	–	–	+	+	v
S. lactativora	–	–	+	–	+	+	+	–	+	–
S. quercuum	s/w	–	–	–	+	+	+	+	–	+

[a] As sole source of nitrogen.

Systematic discussion of the species

49.1. *Sporopachydermia cereana* Rodrigues de Miranda (1978)

Synonym:
Cryptococcus cereanus Phaff, M.W. Miller, Miranda, Heed & Starmer (1974)

Growth on YM agar: After 3 days at 25°C, the cells are ellipsoidal to elongate, (2–4)×(4–14) µm, single, in pairs or in small groups. In some strains, a high proportion of the cells is curved. Growth is butyrous, white to cream-colored, and glistening.

Growth in glucose–yeast extract broth: A sediment and a scant pellicle are formed after 2 weeks at 25°C.

Dalmau plate culture on corn meal agar: A few chains of cells may form under the coverslip after 2 weeks at 17°C.

Formation of ascospores: Asci arise directly from vegetative cells. Asci contain one to four spheroidal to ellipsoidal ascospores with a very thick smooth wall (Fig. 177). The spores are liberated from the ascus at maturity and tend to agglutinate.

Sporulation is scant and rare. Malt extract (1%) and McClary's acetate agar are suitable media. Sporulation is enhanced by transferring cultures from malt extract to acetate medium. Strain CBS 6644 sporulates relatively well after one week on malt extract at 25°C followed by three weeks on acetate at 17°C.

Fermentation: absent.

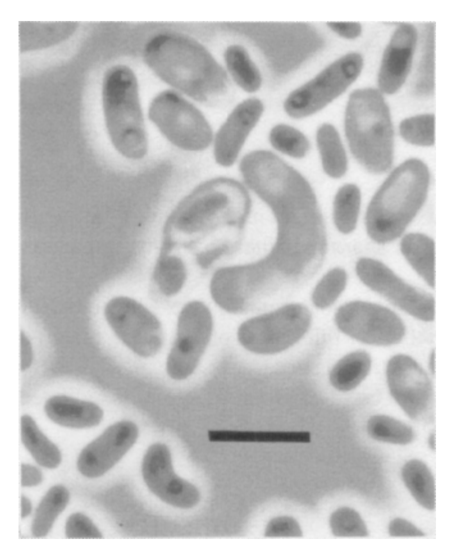


Fig. 177. *S. cereana,* CBS 6644. Vegetative cells and ascus on McClary's acetate agar. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	w/–
L-Arabinose	v	Citrate	–
D-Arabinose	–	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Cycloheximide 100 mg/l	+
Cadaverine	+	1-Butanol	v
L-Lysine	+	Acetone	–
Ethylamine·HCl	+	Tannic acid	–
50% Glucose	–	DBB	–
Gelatin liquefaction	–	Growth at 37°C	+

Co-Q: 9 (Y. Yamada, personal communication).
Mol% G + C: 49.6, CBS 6644 (BD: Phaff et al. 1974).

Origin of the strains studied: UCD-FST 72-313 (CBS 6644), from stem rot of giant saguaro cactus (*Carnegiea gigantea* (Englemann) Britton & Rose), Arizona, U.S.A., is among the many strains isolated from various species of columnar cacti belonging to the subtribe Pachycereinae. Numerous strains are from stem rots of columnar cacti in the subtribe Stenocereinae (e.g., UCD-FST 76-214C, Baja California, Mexico), from cladode rots of prickly pear cacti (*Opuntia* L. spp., e.g., UWO(PS)87-2411.2, Hawaii, U.S.A.), and from necrotic somatic tissue of other cacti (e.g., UCD-FST 76-406C, Sonora, Mexico).

Type strain: CBS 6644, isolated from necrotic somatic tissue of giant saguaro cactus (*Carnegiea gigantea*), Arizona, U.S.A.

Comments: Discrepancies between the above and the information given by Phaff et al. (1974), Rodrigues de Miranda (1978, 1984b), or Lachance (1982a) may be to some extent indicative of the heterogeneous nature of *S. cereana*. Variation occurs between strains in the assimilation of several carbon compounds. We were unable to confirm the weak assimilation of nitrite reported by Rodrigues de Miranda (1984b) for any strain. The problem of variation is complicated by the fact that some, but not all isolates exhibit a characteristic curved cell appearance. This extensive physiological, morphological, and genetic variation does not pattern itself into any evident correlations.

Variation is even more pronounced in yeasts that have been designated in the literature (Lachance et al. 1988, Starmer et al. 1990) as members of the "*S. cereana* complex" because of uncertainties concerning the exact species boundaries of *S. cereana*. Our preliminary results indicate that the complex may represent up to four species, including *S. cereana* sensu stricto. Only strains confirmed as authentic members of the species by DNA reassociation with the type strain were used as a basis for the data reported here. The assimilation of erythritol and the lack of growth on tannic acid (0.5% in Yeast Nitrogen Base, with or without added glucose) are consistent throughout the complex, but variation exists in most other characters.

Representatives of the "*S. cereana* complex" were present in 30% of approximately 1700 samples of necrotic somatic tissue of cacti examined so far by Starmer and coworkers (1990), making it the second (after *Pichia cactophila*) most abundant cactophilic yeast. The conspicuous absence of *S. cereana* from cactus fruit samples has been attributed to the abundance, in cactus fruit, of yeasts capable of producing killer toxins to which certain strains of *S. cereana* are sensitive (Starmer et al. 1987). Moreover, a negative correlation exists between the presence of *S. cereana* in individual samples of prickly pear somatic tissue and the occurrence in each sample of strong killer strains of *Pichia kluyveri*.

49.2. *Sporopachydermia lactativora* Rodrigues de Miranda (1978)

Synonym:
Cryptococcus lactativorus Fell & Phaff (1967)

Growth on YM agar: After 3 days at 25°C, the cells are ellipsoidal to elongate, (2–4) × (4–6) µm, single, in pairs or in small groups. Growth is butyrous, white to cream-colored, and glistening.

Growth in glucose–yeast extract broth: A sediment is formed after 2 weeks at 25°C.

Dalmau plate culture on corn meal agar: Pseudomycelium or true mycelium are not formed after one month at 17°C.

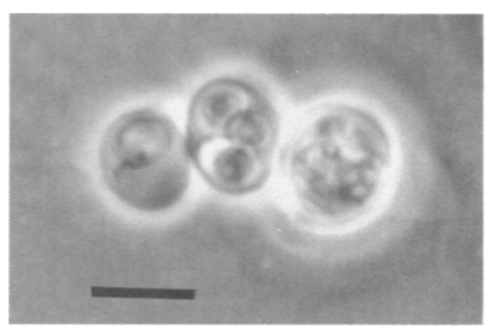


Fig. 178. *S. lactativora*, CBS 6989. Vegetative cells and ascus on McClary's acetate agar. Bar = 5 μm.

Formation of ascospores: Asci arise from the conjugation of cells of opposite mating types, or in diploid strains, directly from vegetative cells. They contain one to four spheroidal ascospores with a very thick smooth wall and an oil droplet inside (Fig. 178). The spores are liberated from the ascus at maturity and tend to agglutinate.

Sporulation is generally scant in mixtures of compatible cells. It is enhanced by transferring cultures showing signs of sexuality to fresh sporulation media. The diploid strain CBS 6989 sporulates abundantly on 1% malt extract and on McClary's acetate agar after 3–5 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Cycloheximide 100 mg/l	+
Cadaverine	–	1-Butanol	+
L-Lysine	+	Acetone	+
Ethylamine·HCl	+	Tannic acid	+
50% Glucose	–	DBB	–
Gelatin liquefaction	–	Growth at 37°C	+

A positive DBB reaction can be obtained in some cases (see Comments on the genus).

Co-Q: 9 (Y. Yamada, personal communication).

Mol% G+C: 46.1–46.3, the latter for CBS 5771, type strain of *Cryptococcus lactativorus*, the former for CBS 5772 (BD: Phaff et al. 1974).

Origin of the strains studied: CBS 5771 and CBS 5772, in two distinct samples of sea water near the Antarctic Peninsula, Fell, U.S.A.; CBS 6058, waste lagoon of asphalt plant, Georgia; CBS 6192, from the mouth of a patient, Finland; CBS 6989, diploid strain selected from a mixture of strains CBS 5771, CBS 5772, and CBS 6192, Rodrigues de Miranda.

Complementary mating types: CBS 6912 and CBS 5771.

Type strain: CBS 6192, from the mouth of a patient; CBS 5771, from Antarctic sea water, is the isotype.

Comments: Rodrigues de Miranda (1984b) designated strain CBS 6989, a diploid selected from a mixture of strains CBS 5771, CBS 5772, and CBS 6192, as the type culture. However, in the original description (Rodrigues de Miranda 1978), strain CBS 6192 had been chosen as the holotype, with CBS 5771 as the isotype. In accordance with Article 7.3, Note 1 of the International Code of Botanical Nomenclature (Greuter et al. 1988), the earlier designation must prevail.

Natural isolates of *S. lactativora* are haploid with their mating compatibility apparently controlled by two alleles of a single locus. Strain CBS 6912 is arbitrarily designated as h^+, equivalent to *a*, following Rodrigues de Miranda's (1978, 1984b) convention, and strains CBS 5771, CBS 5772, and CBS 6058 are of mating type h^- (equivalent to α).

Minor discrepancies exist between our nutritional data and those given by Rodrigues de Miranda (1978, 1984b). When tested by replica plating on agar media, all four strains studied failed to utilize L-arabinose. Strains CBS 6192 and CBS 6989 grew slowly on D-xylose and rapidly on mannitol and glucitol, whereas the other three gave clearly negative responses on these tests. At variance with the responses in Barnett et al. (1983) was our observation, in all strains, of growth on ribitol as carbon source and lack of growth on cadaverine as nitrogen source.

The infrequent isolation of *S. lactativora* precludes the formulation of extensive hypotheses about its ecology, although the species appears to be cosmopolitan in its distribution. Because of its high maximum temperature of growth in contrast to the sea water temperature from which some strains were isolated (ca. 0°C), Fell and Phaff (1967) suggested that its occurrence in sea water could have come from feces of several species of large birds that are common in Antarctica.

49.3. *Sporopachydermia quercuum* Lachance (1982a)

Growth on YM agar: After 3 days at 25°C, the cells are ellipsoidal to elongate, frequently curved, (2–3)×(4–8) μm, single, in pairs or in small groups. Growth is butyrous, white to cream-colored, and glistening.

Growth in glucose–yeast extract broth: A sediment and a very spotty ring are formed after one month at 25°C.

Dalmau plate culture on corn meal agar: A few chains of cells may be formed under the coverslip after 2 weeks at 17°C.

Formation of ascospores: Asci arise directly from vegetative cells or following conjugation between cells within the same culture (Fig. 179). Asci may bear protuberances indicative of bud–parent cell conjugation. Asci contain one to four ovoid, ellipsoid to bacilliform ascospores with a very thick smooth wall. The spores are liberated from the ascus at maturity and tend to agglutinate.

Sporulation is scant on McClary's acetate agar, and is enhanced by transferring cultures exhibiting protuberance formation or conjugation to fresh sporulation medium and incubating at 17°C. All strains are sporogenous, strain CBS 8070 being one of the most vigorous.

Fermentation:

Glucose	s/w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Cycloheximide 100 mg/l	+
Cadaverine	+	1-Butanol	+
L-Lysine	+	Acetone	+
Ethylamine·HCl	+	Tannic acid	+
50% Glucose	–	DBB	–
Gelatin liquefaction	–	Growth at 37°C	–

A positive DBB reaction can be obtained in some cases (see Comments on the genus).

Co-Q: 9 (Y. Yamada, personal communication).

Mol% G+C: 37.8, CBS 8070 (BD: Phaff, unpublished data).

Origin of the strains studied: UWO(PS)80-118 (CBS 8070, ATCC 56629), UWO(PS)80-141 (ATCC 56630) and other strains were collected from slimy exudates of red oaks (*Quercus rubra* L.), Ontario, Canada.

Type strain: CBS 8070, from red oak (*Quercus rubra* L.).

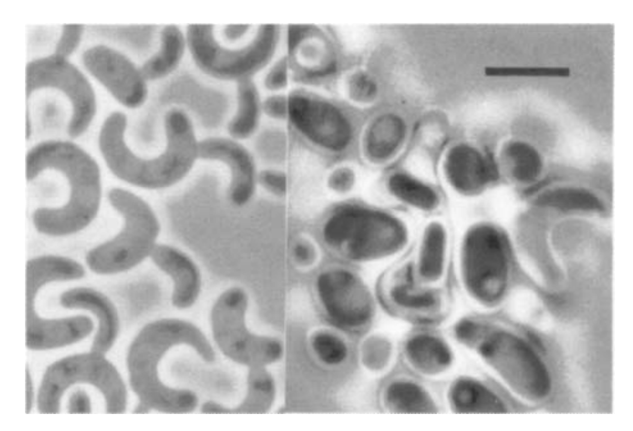


Fig. 179. *S. quercuum*, CBS 8070. Vegetative cells on YM agar. Ascus on McClary's acetate agar (from Lachance 1982a). Bar = 5 μm.

Comments: *S. quercuum* was described (Lachance 1982a) to accommodate isolates from red oak (*Quercus rubra* L.) exudates, whose characteristics differed only slightly from those of *S. lactativora*. The predominance of curved cells and the difference in maximum growth temperature were taken as sufficient grounds for the description of a separate species. This is confirmed by the nearly 8% difference in nDNA base composition with the other two accepted species.

Cultures of *S. quercuum* appear to be mixtures of haploid and diploid cells. Because of the scant sporulation and the tendency of liberated spores to agglutinate, no attempt has been made to determine whether self-fertility is due to homothallism.

A difference exists between the maximum growth temperatures reported by different laboratories (Lachance 1982a, Barnett et al. 1983). As this difference is important for the identification of *S. quercuum*, it is worth mentioning that the measurements reported here were performed on replica plates with both synthetic (YNB–glucose) and complex (YM) agar media. The maximum determined in this way was 34.5±0.5°C. A higher value (36±0.5°C) was obtained on media dispensed in tubes and incubated in a heating block (J.E. Kaden and M.A. Lachance, unpublished results). Barnett et al. (1983), using liquid media, reported good growth at 37°C and no growth at 42°C.

Comments on the genus

Rodrigues de Miranda (1978) described the genus *Sporopachydermia* based on the observation of the formation of thick-walled ascospores in two species of *Cryptococcus* known not to be basidiomycetous anamorphs (Phaff et al. 1974). Although neither the chemistry nor the evolutionary significance of the spore sheath are understood, such a feature brings together species that represent an evolutionarily coherent assemblage. Ribosomal RNA sequence comparisons (Yamada et al. 1992a) confirmed this view, showing that *Sporopachydermia* is monophyletic with respect to various other Q-9 genera. Phenetic analysis of the physiological responses of all yeasts given in Barnett

et al. (1983) shows that the combination of nutritional abilities found in the genus is unique among yeasts with demonstrated ascomycetous life cycles, substantiating the hypothesis that the genus is monophyletic within ascomycetes. A superficial physiological resemblance exists, however, with the asexual species *Trigonopsis variabilis, Candida pararugosa,* and *Candida sorbophila.* The nDNA base compositions of members of these taxa fall well within the range exhibited by *Sporopachydermia* species.

The slow but positive diazonium blue B reaction reported in *S. quercuum* (Lachance 1982b) occurs also in *S. lactativora,* but not in *S. cereana.* A purple reaction is observed after two minutes on colonies grown on casein hydrolysis agar medium. Colonies grown on YM agar and subjected to a 50°C treatment, as recommended by Hagler and Ahearn (1981), gave a yellow reaction (M.A. Lachance, unpublished results). Although surprising, these results are not unparalleled. Summerbell (1985) observed normal (purple) diazonium blue B reactions in several filamentous fungi of ascomycetous affinity, if they were first subjected to an alkali treatment. Yellow reactions occurred in a few basidiomycetous species without alkali treatment. Simmons and Ahearn (1987) examined closely the cell wall structure and the diazonium blue B reaction of *S. quercuum,* and concluded that the yeast is typically ascomycetous for both traits. They suggested that the positive reaction reported by Lachance (1982b) was most likely the result of impurities present in the chemical. While the complete significance of all these observations remains to be clarified, the sharing of such an unusual property by two *Sporopachydermia* species acts as additional evidence of their close relatedness.

Sporopachydermia appears to be ecologically similar to *Clavispora* and *Pichia,* in that these genera contain some species that act as generalists, and others as specialists. The little that is known about *S. lactativora* suggests that it is a generalist. *S. cereana* and *S. quercuum* appear to be specialists, although the small number of isolates of the latter species limits speculation at this time.

50. *Stephanoascus* M.Th. Smith, van der Walt & E. Johannsen

M.Th. Smith and G.S. de Hoog

Diagnosis of the genus

Colonies are restricted, dry, and snow-white. Asexual reproduction is either by multilateral budding (anamorph genus *Candida*) or by short chains of sympodial conidia on true hyphae (anamorph genus *Blastobotrys*).

Gametangia are formed on hyphae after fusion of two intercalary cells or lateral outgrowths. Asci are globose with firm, thick walls and contain 2–4 ascospores. Asci bear a sterile apical cell. Ascospores are oblate to hat- or helmet-shaped. Septa with micropores are present.

Sugars may be fermented. Nitrate may be assimilated. Diazonium blue B reaction is negative.

Type species

Stephanoascus ciferrii M.Th. Smith, van der Walt & E. Johannsen

Species accepted

1. *Stephanoascus ciferrii* M.Th. Smith, van der Walt & E. Johannsen (1976)
2. *Stephanoascus farinosus* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)
3. *Stephanoascus smithiae* Giménez-Jurado (1994)

Key to species

See Table 42.

1. a Apical cell on ascus is hemispherical; growth at 37°C .... *Stephanoascus ciferrii*: p. 400
   b Apical cell on ascus is clavate; growth absent at 37°C → 2

2(1). a Growth with inositol .... *Stephanoascus smithiae*: p. 402
   b Growth absent with inositol .... *Stephanoascus farinosus*: p. 401

Table 42
Key characters of species in the genus *Stephanoascus*

Species	Glucose fermentation	Assimilation		Growth at 37°C	Apical cell	Mating system
		Succinate	Inositol			
Stephanoascus ciferrii	–	+	+	+	hemispherical	heterothallic
S. farinosus	w	–	–	–	clavate	homothallic
S. smithiae	–	+	+	–	clavate	heterothallic

Systematic discussion of the species

50.1. *Stephanoascus ciferrii* M.Th. Smith, van der Walt & E. Johannsen (1976)

Synonyms:
Candida ciferrii Kreger-van Rij (1965)
Sporothrix catenata de Hoog & Constantinescu (1981)
Candida mucifera Kocková-Kratochvílová & Sláviková (1988)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 4 mm in diameter, dry, white, and somewhat raised. Budding cells are absent or present, multilateral, and often in short chains. True hyphae are absent or present, 2–3 µm wide, and form a compact mycelium. Conidia are sympodially produced in dense, swollen clusters; they are terminal or intercalary just below hyphal septa, with marked, sharp denticles bearing conidia. Conidia are formed singly or in short chains about (1.5–3.0)×(3.5–5.5) µm; lower conidia are somewhat larger.

Formation of ascospores: Gametangia are formed by conjugation of opposite hyphal cells; the conjugation bridge forms a lateral outgrowth which develops into an ascus. Asci are globose, with firm, thick, persistent walls, and are crowned by a hemispherical sterile cell. Asci

contain 2–4 oblate to hat-shaped ascospores measuring (3–5)×(5–8) μm (Fig. 180). The species is heterothallic.

Mating tests are conducted on V8 agar at 28°C.

Fermentation: absent.

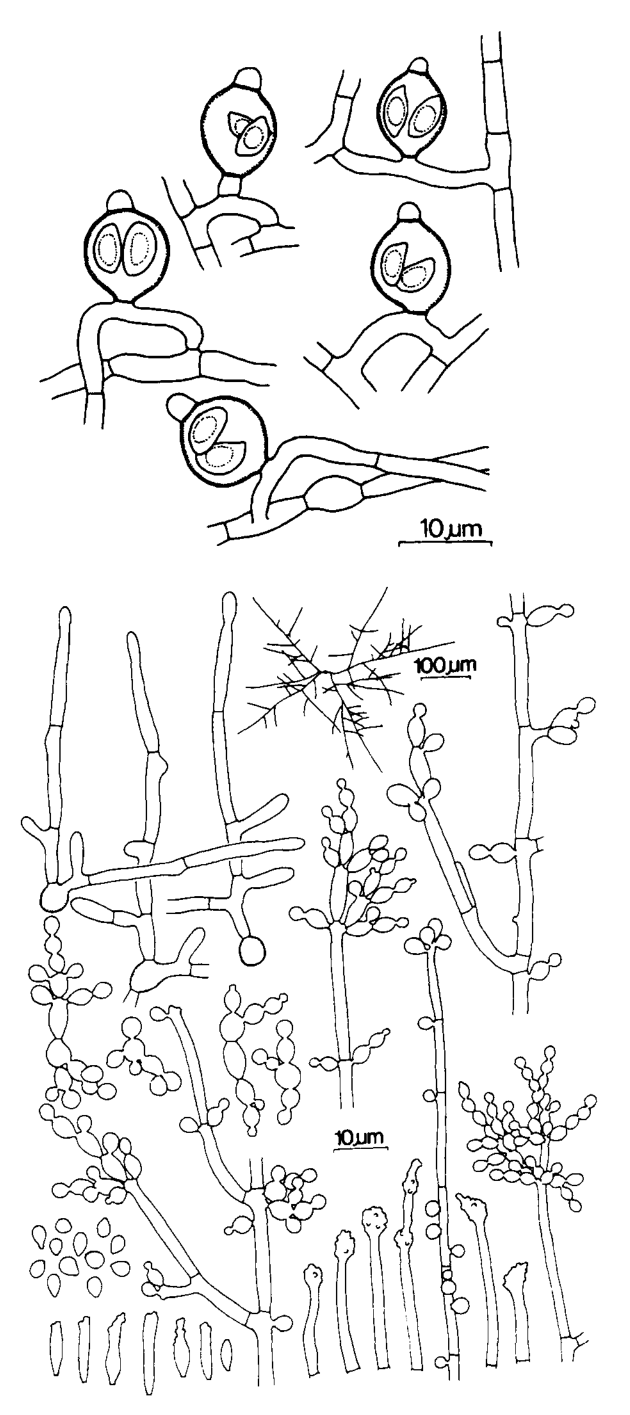


Fig. 180. *S. ciferrii*, CBS 5295×6699. Asci with ascospores (top). Asci are on the conjugation bridge formed from conjugation of hyphae of opposite mating partners. V8, 28°C, 10 days. Anamorph (bottom) (CBS 215.79) producing short, branched chains of conidia from swollen, denticulate heads; clusters of multilateral budding cells. MEYA, 22°C, 10 days.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	v
L-Arabinose	+	Citrate	v
D-Arabinose	+	Inositol	+
D-Ribose	v	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Arbutin	v	Growth at 37°C	+

Co-Q: 9, CBS 5295, CBS 6699 and 4 additional strains (Yamada and Smith 1985).

Mol% G + C: 46.2–47.0, CBS 6699 (T_m: Giménez-Jurado et al. 1994).

Origin of the strains studied: CBS 4856 (ATCC 22873), type strain of *Candida ciferrii,* from a cow, Netherlands; CBS 7409, type strain of *Candida mucifera,* from liver of a toad, Brazil; CBS 215.79, type strain of *Sporothrix catenata,* from calf skin, Romania; from fence post (1); from pig (1); from bovine placenta (1); from soil (1).

Complementary mating types: CBS 5645 (*a*), 6699 (*a*), 7409 (*a*), 4856 (*α*), 5165 (*α*), 5166 (*α*), 5295 (*α*).

Isotype strains: CBS 6699 and CBS 5295.

Comments: The species is often associated with animals and occasionally found in humans, mainly involved in otitis (Furman and Ahearn 1983). It is slightly thermophilic. The type strain of *Candida mucifera* from toad liver in Brazil was found to be mating type *a*, and produces ascospores with the tester strain CBS 5295.

Some strains are strongly hyphal and produce conidia from characteristically inflated, denticulate heads. This anamorph has been described as *Sporothrix catenata.*

50.2. *Stephanoascus farinosus* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)

Synonyms:

?*Sporothrix fungorum* de Hoog & de Vries (1973)

Blastobotrys farinosus de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)

?*Blastobotrys gigas* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 5 mm in diameter, white, and farinose with flat, glassy margins. Expanding hyphae are straight and somewhat stiff, 1.8–2.5 μm wide, forming terminal and intercalary groups of

primary conidia, (3–5)×(5–7) µm, each bearing several globose secondary conidia, 2–3 µm in diameter.

Formation of ascospores: Asci are formed from fused gametangia laterally on hyphae. They are globose, 7–9 µm in diameter, with persistent, firm walls, and are crowned with a cylindrical to clavate top cell. Asci contain 2–4 ascospores that are broadly ellipsoidal, 2.0–2.5 µm, with an irregular gelatinous basal brim, the widest diameter about 4 µm (Fig. 181). The species is homothallic.

Asci are formed on MEYA at 15°C.

Fermentation:

Glucose	w	Lactose	v
Galactose	v	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	v	Ethanol	v
Sucrose	–	Glycerol	+
Maltose	v	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	–
D-Xylose	v	Succinate	v
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Arbutin	v	Growth at 37°C	–

Co-Q: 9, IGC 4592 (Giménez-Jurado et al. 1994).
Mol% G+C: 48.5–49.9, IGC 4592 (T_m: Giménez-Jurado et al. 1994).

Origin of the strains studied: CBS 140.71, from the carpophore of *Hirneola auricula-judae,* Gams, Netherlands; CBS 421.78, type strain of *Blastobotrys gigas,* from a frozen mummy, Bodenhoff, Greenland; CBS 259.70, type strain of *Sporothrix fungorum,* from carpophore of *Fomes fomentarius,* Gams, Germany; from carpophore of *Corticium* (1); from carpophore of *Hirneola* (1); from Sphaeriales (1).

Type strain: CBS 140.71.

Comments: The species is homothallic. Traquair et al. (1988a) reported *S. farinosus* teleomorphs in strains which de Hoog et al. (1985) found to be different in morphology and physiology. However, characters are notoriously variable, as demonstrated by physiological differences between two strains of *Sporothrix fungorum* (de Hoog et al. 1985).

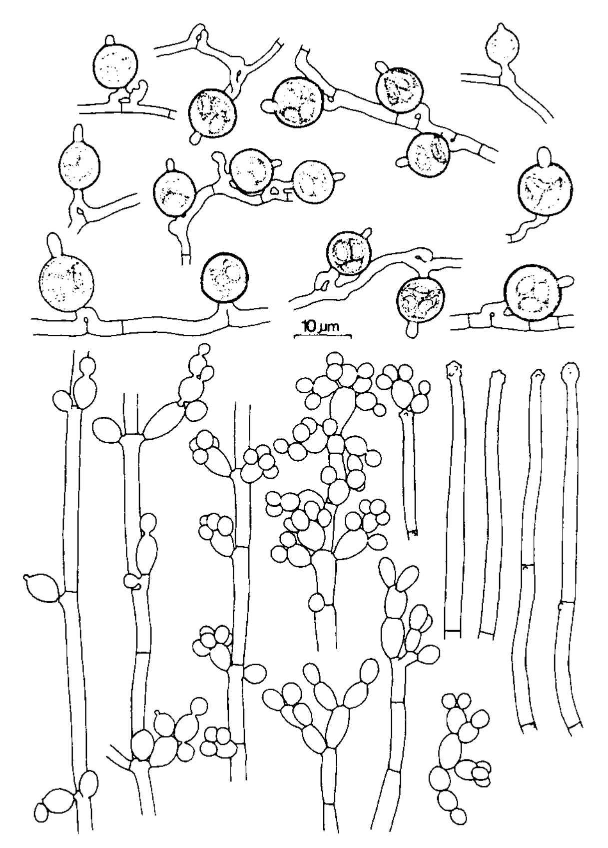


Fig. 181. *S. farinosus,* CBS 140.71. Asci with ascospores (top). Asci are on the conjugation bridge formed from adjacent cells of a single hypha. MEYA, 15°C, 10 days. Anamorph (bottom) with conidia in compact clusters, formed on small denticles. MEYA, 20°C, 10 days.

The species is somewhat psychrophilic, with asci developing at 18°C. It is found on dead carpophores of fungi, be they basidio- or ascomycetous. *Blastobotrys gigas* was isolated from a mummy in ice (Bodenhoff et al. 1979); it differs markedly from the remaining anamorphs by having large conidioferous denticles. De Hoog et al. (1985), though with hesitation, introduced separate names for the groups within this taxonomic complex. DNA/DNA reassociations are needed to determine species borderlines and to evaluate currently used taxonomic criteria in these hyphal yeasts.

50.3. *Stephanoascus smithiae* Giménez-Jurado (Giménez-Jurado et al. 1994)

Synonym:
Candida edax van der Walt & Nel (1968)

Growth on 2% glucose/0.5% yeast extract/1% peptone agar: After 7 days at 20–22°C, colonies are white to cream-colored, compact, and somewhat raised at the center. Budding cells are scarce, multilateral and measure (1–2)×(2–12) µm. True hyphae are present, with conidia produced from denticle-like structures, either terminal or intercalary (Fig. 182). Conidia measure about (1.5–4.0)×(2.0–3.0) µm.

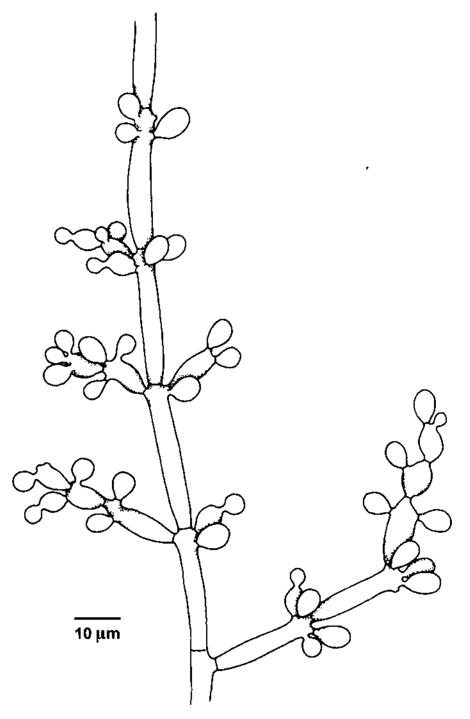


Fig. 182. *S. smithiae*, CBS 7522.1. Anamorph with conidia formed on denticulate heads. MEYA, 10°C, 10 days.

Formation of ascospores: Gametangia are formed by conjugation of opposite hyphal cells, the conjugation bridge forms a lateral outgrowth which develops into an ascus. Asci are globose, with firm, thick, persistent walls, (5.0–8.0)×(3.0–5.0) µm, and are crowned with a sterile clavate cell. Asci contain one hemispherical or helmet-shaped ascospore which has a thick basal brim (Fig. 183). The species is heterothallic.

Mating tests are conducted on malt extract agar at 25°C.

Fermentation: absent.

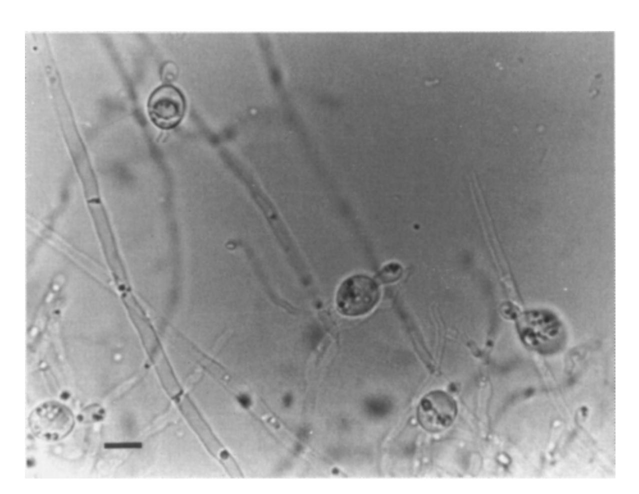


Fig. 183. *S. smithiae*, CBS 7522.1×7522.2. Asci with ascospores on oatmeal agar, 1 week, 25°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+/w	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

Arbutin	+	Hydrolysis of urea	–
Nitrite	+	0.01% Cycloheximide	+
Cadaverine	+	0.1% Cycloheximide	+
Creatinine	–	Growth with Tween 80	–
Creatine	–	Growth with 5% olive oil	–
Ethylamine	+	Growth at 37°C	+
Starch formation	–		

Co-Q: 9, CBS 7522.1, 7522.2 (Giménez-Jurado et al. 1994).

Mol% G+C: 47.0–47.4, CBS 7522.1, 7522.2 (T_m: Giménez-Jurado et al. 1994).

Origin of the strains studied: CBS 5657 (ATCC 18414, IFO 10273, NRRL Y-17083), type strain of *Candida edax*, from an insect tunnel, South Africa, van der Walt; CBS 6461, from soil, Japan, Furukawa; CBS 7522.1 (IGC 4646) and 7522.2 (IGC 4647), isotype strains of *Stephanoascus smithiae*, from soil, Brazil.

Complementary mating types: CBS 7522.1 and CBS 7522.2.

Isotype strains: CBS 7522.1 (mt *a*) and CBS 7522.2 (mt *α*).

Comments: A full description of the species was given by Giménez-Jurado et al. (1994), including results of an extended series of assimilation tests. The species was shown to have one central micropore in the hyphal septa. Its identity as a new species was proven by low DNA/DNA homology values with the remaining *Stephanoascus* species.

Comments on the genus

Stephanoascus species are variable in the morphology of their anamorphs and in their physiology. They may be hyphal, with characteristic, sympodial conidiogenous apparatus, or entirely yeastlike.

Currently three species are accepted. The authentic strains of *S. flocculosus* Traquair et al. (1988b) and *S. rugulosus* Traquair et al. (1988b) proved to be *Pseudozyma* anamorphs of smut fungi (Boekhout 1995).

51. *Torulaspora* Lindner

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal to ellipsoidal. Pseudohyphae may be present but true hyphae are not formed.

Asci are persistent and may be unconjugated or show conjugation between a cell and its bud or between independent cells. Cells with tapered protuberances resembling conjugation tubes may also be present. Asci contain 1–4 spheroidal ascospores that are either roughened or smooth.

Sugars are fermented. Nitrate is not assimilated. Pellicles are absent or weakly formed. Coenzyme Q-6 is produced. Diazonium blue B reaction is negative.

Type species

Torulaspora delbrueckii (Lindner) Lindner

Species accepted

1. *Torulaspora delbrueckii* (Lindner) Lindner (1904)
2. *Torulaspora globosa* (Klöcker) van der Walt & E. Johannsen (1975)
3. *Torulaspora pretoriensis* (van der Walt & Tscheuschner) van der Walt & E. Johannsen (1975)

Key to species

See Table 43.

1. a Growth at 37°C strong and rapid → 2
   b Growth at 37°C slow, weak or absent .... *T. delbrueckii*: p. 404
2(1). a Galactose assimilated .... *T. pretoriensis*: p. 406
   b Galactose not assimilated .... *T. globosa*: p. 406

Table 43
Key characters of species in the genus *Torulaspora*

Species	Assimilation			Growth	
	Galactose	Maltose	α-Methyl-D-glucoside	0.01% Cycloheximide	at 37°C
Torulaspora delbrueckii	v	v	v	–	s,w,–
T. globosa	–	–	–	×	+
T. pretoriensis	+	+	+	–	+

Systematic discussion of the species

51.1. *Torulaspora delbrueckii* (Lindner) Lindner (1904)

Anamorph: *Candida colliculosa* (Hartmann) S.A. Meyer & Yarrow; on the basis of phenotypic similarity.

Synonyms:
Saccharomyces delbrueckii Lindner (1895)
Zygosaccharomyces delbrueckii (Lindner) Krumbholz (1933)
Debaryomyces delbrueckii (Lindner) Kudryavtsev (1954)
Zymodebaryomyces delbrueckii (Lindner) Novák & Zsolt (1961)
Torula colliculosa Hartmann (1903)
Torulopsis colliculosa (Hartmann) Saccardo (1906)
Eutorula colliculosa (Hartmann) Will (1916)
Cryptococcus colliculosus (Hartmann) Skinner (1950)
Candida colliculosa (Hartmann) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Torulaspora rosei Guilliermond (1913)
Saccharomyces rosei (Guilliermond) Lodder & Kreger-van Rij (1952)
Debaryomyces rosei (Guilliermond) Kudryavtsev (1954)
Zymodebaryomyces rosei (Guilliermond) Novák & Zsolt (1961)
Torulaspora fermentati Saito (1923)
Zygosaccharomyces fermentati (Saito) Krumbholz (1933) [non *Zygosaccharomyces fermentati* Naganishi (1928)]
Saccharomyces fermentati (Saito) Lodder & Kreger-van Rij (1952)
Zygosaccharomyces mongolicus Saito (1923)
Saccharomyces delbrueckii Lindner var. *mongolicus* (Saito) Lodder & Kreger-van Rij (1952)
Torulaspora mongolica (Saito) van der Walt & E. Johannsen (1975a)
Saccharomyces torulosus Osterwalder (1924a)
Saccharomyces chevalieri Guilliermond var. *torulosus* Dekker (Stelling-Dekker 1931)
Zygosaccharomyces globiformis Kroemer & Krumbholz (1931)
Torulopsis cambresieri Monoyer (Lodder and Kreger-van Rij 1952)
Torulopsis stellata (Kroemer & Krumbholz) Lodder var. *cambresieri* Lodder & Kreger-van Rij (1952)
Torulaspora nilssoni Capriotti (1958b)
Saccharomyces nilssoni (Capriotti) Santa María (1963b)

Debaryomyces nilssoni (Capriotti) K. Kodama, Kyono, Naganishi & Takahara (1964c)
Schwanniomyces hominis Batista, Vieira & Coêlho (1959b)
Debaryomyces dekkeri Mrak, Phaff, Vaughn & Hansen ex Kudryavtsev (1960)
Zymodebaryomyces dekkeri (Mrak, Phaff, Vaughn & Hansen ex Kudryavtsev) Novák & Zsolt (1961)
Saccharomyces inconspicuus van der Walt (1965a)
Torulaspora inconspicua (van der Walt) van der Walt & E. Johannsen (1975a)
Saccharomyces vafer van der Walt (1965a)
Torulaspora vafer (van der Walt) van der Walt & E. Johannsen (1975a)
Torulaspora benedictae Capriotti (1969)
Saccharomyces saitoanus van der Walt (1970c)
Saccharomyces microellipsodes Osterwalder var. *osmophilus* van der Walt (1970c)
Saccharomyces florenzani Balloni, Materassi & Margheri (1971)
Torulopsis taboadae Ulloa & Herrera (1978)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.1–5.6)×(3.0–6.6) μm, and single or occasionally in pairs. Growth is butyrous, dull-glistening and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass may show a few chains of somewhat elongated cells, but there are no pseudohyphae or true hyphae. Aerobic growth is white to tannish-white, smooth, moderately glistening, and butyrous. Colonies are low convex with a depressed center and a smooth margin. A faint acidic odor is present.

Formation of ascospores: Asci are persistent and often conjugated. Conjugation may be between a cell and its bud or between independent cells. In addition, cells with tapered protuberances that resemble conjugation tubes are often present. Asci form one to four spheroidal ascospores that often appear faintly roughened under the light microscope (Fig. 184).

Ascospores were observed on YM, 5% malt extract, corn meal, McClary's acetate and RG agars after 5–30 days at 15 or 25°C.

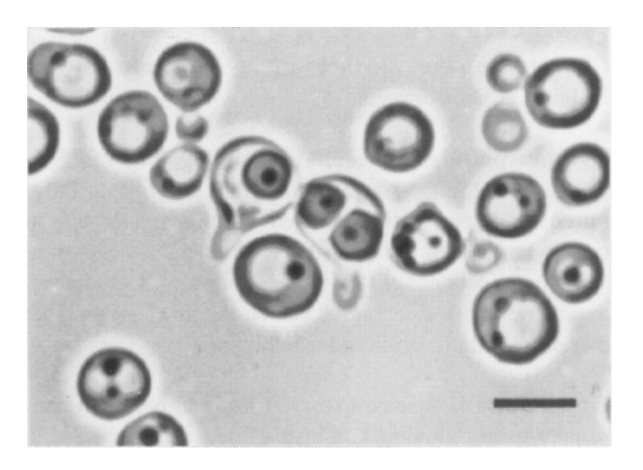


Fig. 184. *T. delbrueckii*, CBS 4865. Asci with ascospores, after 3 weeks on RG agar at 15°C. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	v
Sucrose	v	Trehalose	v
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	v	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	–
Inulin	v	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	v	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	0.01% Cycloheximide	–
Cadaverine	–	Growth at 37°C	v
10% NaCl/5% glucose	+		

Co-Q: 6, CBS 1146, and numerous other strains (Yamada et al. 1976b).
Mol% G + C: 42.7–43.9, CBS 1146, and numerous other strains, (T_m: Nakase and Komagata 1971e); 43.4–43.9 (BD: Price et al. 1978).

Origin of the strains studied: Cucumber brine, North Carolina, U.S.A. (5); fermented sausages, U.S.A. (2); food processing plant, U.S.A. (1); ragi, Indonesia (2); sugar mill (1); sorghum brandy (1); type strain of *Torulopsis taboadae*, wine, Mexico; pickled herring, U.S.A. (1); grape juice (1); spoiled beer (1); insect frass, U.S.A. (2); soil, U.S.A. (2), Ghana (1); slime flux, oak (*Quercus* sp.), U.S.A. (1); rotted tree stumps, eastern U.S.A. (3); unknown (4).

Type strain: CBS 1146 (NRRL Y-866), neotype designated by van der Walt (1970d).

Comments: Strains of *T. delbrueckii* show considerable variation in their abilities to ferment and to assimilate carbon compounds, which has contributed to the uncertain circumscription of this species and has resulted in the description of numerous taxa that are now regarded to be synonyms. Price et al. (1978) determined the extent of nDNA complementarity among many of the described species of *Torulaspora* and demonstrated that such long recognized species as *T. rosei*, *T. fermentati*, and *T. vafer* are conspecific with *T. delbrueckii*. However, not all of the taxa listed as synonyms have been subjected to nDNA comparisons.

51.2. *Torulaspora globosa* (Klöcker) van der Walt & E. Johannsen (1975a)

Synonyms:
Debaryomyces globosus Klöcker (1909a)
Zymodebaryomyces globosus (Klöcker) Novák & Zsolt (1961)
Saccharomyces kloeckerianus van der Walt (1971)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (3.2–6.6)×(3.3–6.6) µm, and single or in pairs. Growth is butyrous, dull-glistening, and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is white to tannish-white, smooth, moderately glistening, and butyrous. Colonies are low convex with a depressed center and an entire to lobed margin. A faintly acidic odor is present.

Formation of ascospores: Asci are persistent and usually form following conjugation between individual cells, or less often between a cell and its bud. Cells bearing protuberances are often present. Asci form one or two spheroidal ascospores that often appear faintly roughened when viewed with the light microscope (Fig. 185). Yarrow (1984c) reported the presence of mating types.

Ascospores were observed on YM and RG agars after 5–10 days at either 15 or 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+/w
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	v	D-Gluconate	w/–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	0.01% Cycloheximide	+/w
Cadaverine	–	0.1% Cycloheximide	–
10% NaCl/5% glucose	v	Growth at 37°C	+

Co-Q: 6, CBS 764, and 4 additional strains (Yamada et al. 1976b).
Mol% G + C: 47.5, CBS 764; 47.0, CBS 5380 (BD: Price et al. 1978).

Origin of the strains studied: Soil, West Indies (1), New Guinea (1), unknown (2).

Complementary mating types: CBS 5500 and CBS 5503.

Type strain: CBS 764 (NRRL Y-12650), from soil, West Indies.

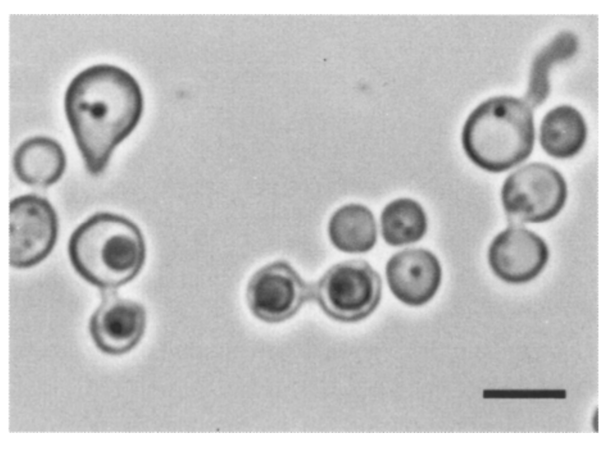


Fig. 185. *T. globosa*, CBS 5380. Conjugated ascus with ascospores, after 7 days on RG agar at 25°C. Bar = 5 µm.

51.3. *Torulaspora pretoriensis* (van der Walt & Tscheuschner) van der Walt & E. Johannsen (1975a)

Synonyms:
Saccharomyces pretoriensis van der Walt & Tscheuschner (1956c)
Torulaspora franciscae Capriotti (1958a)
Debaryomyces franciscae (Capriotti) K. Kodama, Kyono, Naganishi & Takahara (1964b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to short ellipsoidal, (2.5–5.1)×(3.0–6.6) µm, and single or in pairs. Growth is butyrous, dull-glistening, and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows infrequent, poorly differentiated pseudohyphae, but no true hyphae. Aerobic growth is white to tannish-white, smooth, moderately glistening, and butyrous. Colonies are low convex with a depressed center and an entire to lobed margin. A faintly acidic odor is present.

Formation of ascospores: Asci are persistent and usually form following conjugation between a cell and its bud. Each ascus forms one to four spheroidal ascospores that may be roughened or smooth (Fig. 186).

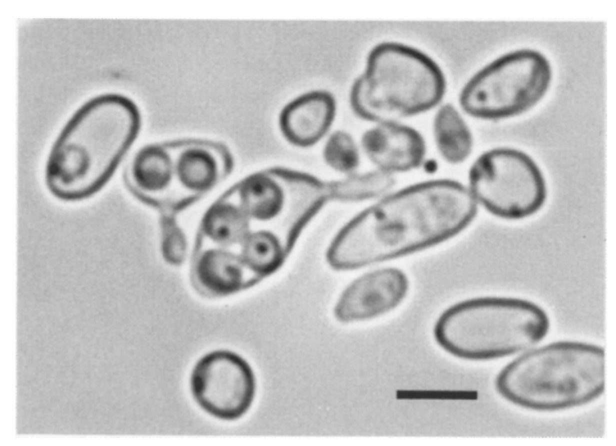


Fig. 186. *T. pretoriensis*, CBS 2926. Conjugated asci with ascospores, after 3 months on YM agar at 5°C. Bar = 5 μm.

Ascospores were observed on YM agar, often following refrigeration (ca. 5°C) for one to several months.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/w	Raffinose	v
Sucrose	+	Trehalose	v
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+/w
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	–
Inulin	+	D-Gluconate	v
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	0.01% Cycloheximide	–
Cadaverine	–	Growth at 37°C	+
10% NaCl/5% glucose	w/–		

Co-Q: 6, CBS 2926 (Yamada et al. 1976b).
Mol% G + C: 45.6, CBS 2926 (T_m: Nakase and Komagata 1971e); 46.4, CBS 2187; 46.3, CBS 2926 (BD: Price et al. 1978).

Origin of the strains studied: CBS 2187 (NRRL Y-17251), from soil, South Africa; CBS 2926 (NRRL Y-17532), type strain of *Torulaspora franciscae,* from soil, Spain.

Type strain: CBS 2187.

Comments on the genus

As shown by the long lists of synonyms for some species, use of phenotypic characters to separate the genera *Torulaspora, Saccharomyces, Zygosaccharomyces* and *Debaryomyces* has given uncertain results. In the 1970 edition of The Yeasts, A Taxonomic Study, *Debaryomyces* was maintained, but species of *Torulaspora* and *Zygosaccharomyces* were assigned to *Saccharomyces.* In 1975, van der Walt and Johannsen revived *Torulaspora* and enlarged it with the transfer of all *Debaryomyces* species and further included species of *Pichia* with spheroidal ascospores. With the 1984 edition of The Yeasts, all four genera were again recognized. Distinguishing characters included the following:

Debaryomyces – Cells are predominately haploid with ascosporulation usually preceded by conjugation between independent cells or between a cell and its bud; ascospores are roughened; fermentation is absent or usually not vigorous; coenzyme Q-9.

Saccharomyces – Cells of some species are predominantly diploid, others are haploid; conjugation may not immediately precede ascosporulation; ascospores are smooth; fermentation is vigorous; coenzyme Q-6.

Torulaspora – Cells are predominantly haploid with ascosporulation usually preceded by conjugation between independent cells or between a cell and its bud; ascospores are usually roughened; fermentation is vigorous; coenzyme Q-6.

Zygosaccharomyces – Cells are predominantly haploid with ascosporulation usually preceded by conjugation between independent cells, often with each conjugant producing ascospores; ascospores are smooth; fermentation is vigorous; coenzyme Q-6.

Examination of ascospores by transmission electron microscopy (Kawakami 1958, Kodama et al. 1964a–c, Kreger-van Rij 1969c, Kreger-van Rij and Veenhuis 1976b) and scanning electron microscopy (Kurtzman et al. 1972, 1975) demonstrated some differences in the patterns of wall ornamentation among rough-spored species, but did not provide a clear separation of genera. Furthermore, one strain of *Torulaspora pretoriensis* had rough ascospores whereas spores of the type strain were smooth. Strains of the other two species of *Torulaspora* always formed roughened ascospores.

Biochemical comparisons, such as the formation of intracellular β-glucosidases (Fiol 1973) and nitrate reductase (Fiol and Billon-Grand 1977), did not clearly resolve genera. The type of coenzyme Q synthesized provided some correlation with genus circumscriptions (Yamada et al. 1976b). Species of *Debaryomyces* have Q-9, whereas *Saccharomyces, Torulaspora* and *Zygosaccharomyces* are characterized by Q-6.

Ribosomal RNA/rDNA sequence comparisons have had the most significant impact on resolving intra- and interspecific relationships among the four genera

(Kurtzman and Robnett 1991, Yamada et al. 1991b, James et al. 1994b, 1996, 1997). From what is presently known, *Debaryomyces* is phylogenetically separate from the other three genera. *Torulaspora* species arise from within *Zygosaccharomyces,* suggesting *Torulaspora* to be an artificial taxon. Although there appears to be poor separation between *Saccharomyces* and *Zygosaccharomyces* (James et al. 1997, Kurtzman and Robnett, unpublished data), the present four genera have been retained until further analyses can be made.

52. *Wickerhamia* Soneda

H.J. Phaff and M.W. Miller

Diagnosis of the genus

Cells are ovoidal to elongate or apiculate, single, in pairs or very short chains, reproducing mainly by bipolar budding on a relatively broad base.

Asci are formed without prior conjugation. Ascospores are cap-shaped; the crown deflects to one side of a sinuous brim, giving the appearance of a sporting cap; usually only one or two spores form per ascus but occasionally there may be many (up to 16 have been reported); the asci rupture when the spores are mature.

Sugars are fermented; nitrate is not assimilated; biotin is the only vitamin required for growth. In liquid media a sediment and a ring are formed. Diazonium blue B reaction is negative.

Type species

Wickerhamia fluorescens Soneda

Species accepted

1. *Wickerhamia fluorescens* Soneda (1960)

Systematic discussion of the species

52.1. *Wickerhamia fluorescens* Soneda (1960)

Anamorph: *Kloeckera fluorescens* Soneda
Synonym:
Kloeckera fluorescens Soneda (1959)

Growth in 5% malt extract: After two or three days at 25°C, cells are apiculate with blunt ends, ovoidal, elongate (sometimes sausage-shaped or slightly curved), or in the shape of bowling pins, (4–7)×(8–15–22) μm. They occur singly, in pairs, and occasionally in groups of three; reproduction is by bipolar budding on a broad base (Fig. 187); a sediment and a thin ring begin to form. After 3–4 weeks there are a sediment, ring and sometimes a few islets, but no pellicle.

Growth on 10% malt agar: After 3–4 weeks the streak culture is gray to slightly yellowish, smooth, semiglossy, texture pasty, cross section low convex to flat, margin entire. The medium may become yellowish due to excretion of riboflavin, which shows strong fluorescence under ultraviolet light.

Dalmau plate culture on potato agar: Pseudomycelium is not formed.

Formation of ascospores: Spores are formed without immediately preceding conjugation of vegetative cells. The ascospores resemble a sporting cap; the crown is deflected to one side of a sinuous brim, and they contain a single, conspicuous refractile lipid globule (Fig. 188).

In our study only one and rarely two spores per ascus have been observed, although Soneda (1960) reported up to 16 per ascus. Upon maturity, asci rupture along an equatorial plane, releasing the spores.

Sporulation is best on corn meal agar, oatmeal agar, or potato agar at 25°C, after 3 to 5 days.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–	Melibiose	–

Fig. 187. *W. fluorescens*, CBS 4565. Cells grown on 5% malt extract for 6 days. Bar = 20 μm.

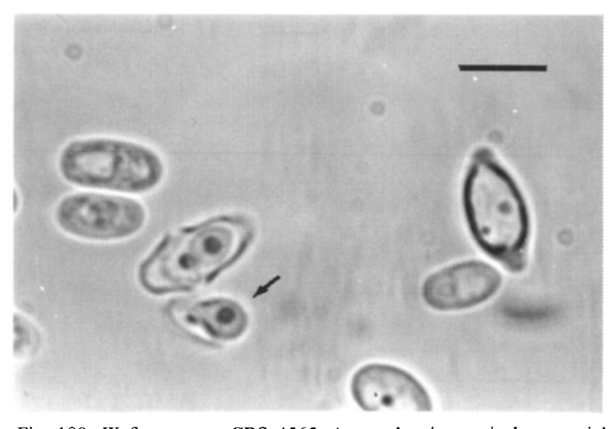


Fig. 188. *W. fluorescens*, CBS 4565. Ascus showing typical equatorial rupture. Arrow indicates liberated ascospore. Bar = 10 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	w
Soluble starch	–	DL-Lactate	w
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Acid production	+
5-Keto-D-gluconate	–	Urease	–
Cadaverine	+	Lipase	–
L-Lysine	+	Gelatin hydrolysis	–
Ethylamine	+	Casein hydrolysis	–
Nitrite	–	Cycloheximide 100 μg/ml	+
50% Glucose	+	Growth at 30°C	+
12.5% NaCl/5% glucose	+	Growth at 37°C	–
Starch synthesis	–		

Co-Q: 9, IFO 1116 (CBS 4565) (Yamada et al. 1976a).

Mol% G+C: 37.6, one strain (T_m: Nakase and Komagata 1971g).

Origin of the strain studied: CBS 4565, isolated by Soneda (1959) from dung of a wild squirrel in Japan.

Type strain: CBS 4565 (IFO 1116).

Comments on the genus

Mitotic division of the rather large nucleus of *W. fluorescens* under phase contrast and in stained preparations has been followed in detail by Matile et al. (1969). Meiosis and ascospore delimitation were studied by Rooney and Moens (1973a,b) with the use of electron microscopy of ultrathin sections. The nuclear behavior is similar to that occurring in *Saccharomyces cerevisiae* in that the nuclear envelope remains intact during the two meiotic divisions and a four-lobed nucleus is formed. One of the four nuclear lobes has a complex spindle-pole body, which is the site from where the prospore wall develops that eventually surrounds the ascospore nucleus and associated cytoplasm. With few exceptions (two-spored asci) the three remaining nuclei degenerate. The outer membrane of the prospore wall forms a fold, or brim, on the outside of the spore, ultimately resulting in an asymmetric cap-shaped spore. Because in the above-cited work and in our own laboratory multispored asci were never observed, it is possible that Soneda's (1960) observation of multispored asci was based on viewing clumps or aggregates of numerous liberated spores.

53. *Wickerhamiella* van der Walt

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on either a narrow or a broad base, and the cells range from spheroidal to ellipsoidal. Neither hyphae nor pseudohyphae are produced. Pellicles are not formed on liquid media.

Asci are conjugated and usually form a single, roughened, asymmetrical elongated ascospore. Asci become deliquescent at the terminus, and following ascospore release, become somewhat corrugated in appearance.

Sugars are not fermented. Nitrate is assimilated. Diazonium blue B reaction is negative.

Type species

Wickerhamiella domercqiae van der Walt

Species accepted

1. *Wickerhamiella domercqiae* van der Walt (1973)

Systematic discussion of the species

53.1. *Wickerhamiella domercqiae* van der Walt (van der Walt and Liebenberg 1973a)

Anamorph: *Candida domercqiae* (van der Walt & van Kerken) S.A. Meyer & Yarrow

Synonyms:

Torulopsis domercqiae (as *T. domercqii*) van der Walt & van Kerken (1960)

Candida domercqiae (van der Walt & van Kerken) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Torulopsis saccharum Shehata (1960) nom. nud.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.2–3.5)×(1.4–3.5) µm, and single, in pairs or in small clusters. CBS 4351 has quite small cells that tend to be at the lower end of the size range given, whereas CBS 4733 has noticeably larger cells. Growth is butyrous and light tannish-white in color.

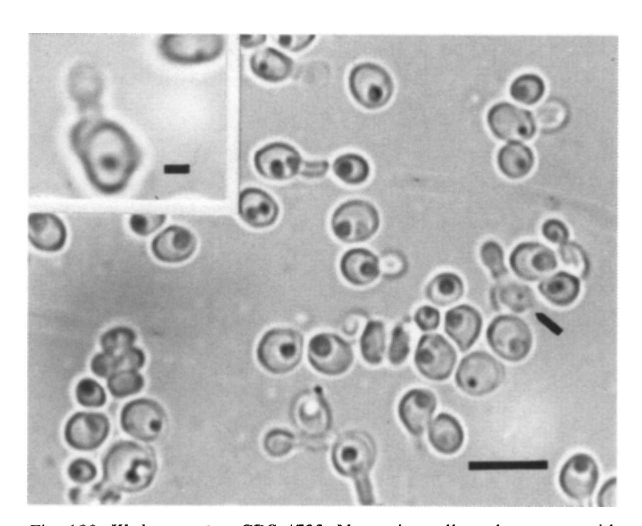


Fig. 189. *W. domercqiae*, CBS 4733. Vegetative cells and an ascus with a single ascospore (arrow), after two weeks on PDA, 15°C. A cluster of four free ascospores is located on the left of the photomicrograph. Bar = 5 µm. Inset. An enlarged view of an ascus with an ascospore. Bar = 1 µm.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither hyphae nor pseudohyphae are seen under the coverglass. Aerobic growth is tannish-white, glistening and butyrous. Colonies are somewhat raised but with a depressed center, and have smooth to finely lobed margins.

Formation of ascospores: Asci are conjugated and each produces a single elongated spore which is released at maturity (Fig. 189). Because of the small size of cells and spores, van der Walt and Liebenberg (1973a) examined the process of ascosporulation by transmission electron microscopy. They demonstrated that asci arise from conjugation between independent cells rather than from parent cell–bud conjugation. The elongated ascospores were shown to be somewhat roughened and irregular in shape. Following dissolution of a terminal portion of the ascus wall, van der Walt and Liebenberg (1973a) stated that the spores are ejected. Empty asci often have a corrugated appearance. Heat treatment of sporulating cultures gave only sporogenous colonies, thus indicating the species to be homothallic.

Ascospores were observed in PDA cultures of CBS 4733 after two weeks at 15°C. Sporulation was not detected in CBS 4351, the type strain.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	+/w
Sucrose	–	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	w
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	s		

Co-Q: Not determined.
Mol% G+C: 48.6, CBS 4351 (BD, T_m: Stenderup et al. 1972).

Origin of the strains studied: NRRL Y-6692 (CBS 4351), type strain of *Torulopsis domercqiae,* from a wine vat, South Africa; NRRL Y-6698 (CBS 4733), type strain of *Torulopsis saccharum,* effluent from a sugar cane factory.

Type strain: CBS 4351, isolated by van der Walt and van Kerken from the surface of a wine vat, South Africa.

Comments on the genus

Wickerhamiella has been isolated only rarely and forms vegetative cells that are among the smallest known for yeasts. The genus is also unique by virtue of its ascospores which have an elongated, irregular shape with a roughened surface.

54. *Williopsis* Zender

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or occasionally elongate. True hyphae are not produced, but pseudohyphae may form. Pellicles are formed on the surface of liquid media by some species.

Asci may be unconjugated or there may be conjugation between a cell and its bud or between independent cells. Asci, which may be either persistent or deliquescent, form 1–4 saturnoid ascospores.

Glucose is fermented. D-xylose is assimilated. Diazonium blue B reaction is negative.

Type species

Williopsis saturnus (Klöcker) Zender

Species accepted

1. *Williopsis californica* (Lodder) von Arx (1977)
2. *Williopsis mucosa* (Wickerham & Kurtzman) Kurtzman (1991)
3. *Williopsis pratensis* Bab'eva & Reshetova (1979)
4. *Williopsis salicorniae* Hinzelin, Kurtzman & M.Th. Smith (1991)
5. *Williopsis saturnus* (Klöcker) Zender (1925)
   a. *Williopsis saturnus* (Klöcker) Zender var. *saturnus* (1991)
   b. *Williopsis saturnus* var. *mrakii* (Wickerham) Kurtzman (1991)
   c. *Williopsis saturnus* var. *sargentensis* (Wickerham & Kurtzman) Kurtzman (1991)
   d. *Williopsis saturnus* var. *suaveolens* (Klöcker) Kurtzman (1991)
   e. *Williopsis saturnus* var. *subsufficiens* (Wickerham) Kurtzman (1991)

Key to species

See Table 44.

1.	a	Cellobiose assimilated → 2		
	b	Cellobiose not assimilated	*W. salicorniae*:	p. 416
2(1).	a	Galactose assimilated	*W. pratensis*:	p. 415
	b	Galactose not assimilated → 3		
3(2).	a	L-Sorbose assimilated → 4		
	b	L-Sorbose not assimilated → 5		
4(3).	a	Melezitose assimilated	*W. mucosa*:	p. 415
	b.	Melezitose not assimilated	*W. californica*:	p. 413
5(3).	a	Sucrose assimilated → 6		
	b	Sucrose not assimilated → 9		
6(5).	a	Growth in vitamin-free medium → 7		
	b	Absence of growth in vitamin-free medium	*W. saturnus* var. *subsufficiens*:	p. 417
7(6).	a	Maltose assimilated	*W. saturnus* var. *saturnus*:	p. 416
	b	Maltose not assimilated → 8		
8(7).	a	L-Rhamnose assimilated	*W. saturnus* var. *saturnus*:	p. 416
	b	L-Rhamnose not assimilated	*W. saturnus* var. *suaveolens*:	p. 417
9(5).	a	Growth in vitamin-free medium	*W. saturnus* var. *mrakii*:	p. 417
	b	Absence of growth in vitamin-free medium	*W. saturnus* var. *sargentensis*:	p. 417

Systematic discussion of the species

54.1. *Williopsis californica* (Lodder) von Arx (von Arx et al. 1977)

Synonyms:
Zygohansenula californica Lodder (1932)
Hansenula californica (Lodder) Wickerham (1951)
Zygowilliopsis californicus (Lodder) Kudryavtsev (1960)
Hansenula californica (Lodder) Wickerham var. *maltosa* Capriotti (1959) nom. nud.
Endomycopsis fukushimae Soneda (1962)
Pichia saturnospora Soneda (1962)
Hansenula dimennae Wickerham (1969b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.4–6.2)×(3.0–8.1) μm, and single or in pairs. Growth is butyrous and gray-white in color.

Growth on the surface of assimilation media: Thin pellicles, or rarely, no pellicles.

Dalmau plate culture on morphology agar: After

Table 44
Key characters of species in the genus *Williopsis*

Species/variety	Fermentation[a]			Assimilation[a]								Vfree[b]
	Glu	Su	Raf	Gal	LSor	Su	Mal	Cel	Mel	LRham	NO_3	
Williopsis californica	+	–	–	–	+	v	v	+	–	v	+	–
W. mucosa	+	–	–	–	+	+	+	+	+	–	–	–
W. pratensis	+	w	–	+	–	+	+	+	+	–	+	–
W. salicorniae	+	–	–	–	–	–	–	–	–	–	–	–
W. saturnus var. *saturnus*[c]	+	+	+/w	–	–	+	v	+	v	v	+	+
W. saturnus var. *mrakii*	+	–	–	–	–	–	–	+	–	v	+	+
W. saturnus var. *sargentensis*	+	–	–	–	–	–	–	+	–	+	–	–
W. saturnus var. *suaveolens*[c]	+	+	+/w	–	–	+	–	+	–	–	+	+
W. saturnus var. *subsufficiens*	+	+	+	–	–	+	–	+	–	+	+	–

[a] Abbreviations: Glu, glucose; Su, sucrose; Raf, raffinose; Gal, galactose; LSor, L-sorbose; Mal, maltose; Cel, cellobiose; Mel, melezitose; LRham, L-rhamnose; NO_3, nitrate.
[b] Growth in vitamin-free medium.
[c] Separation of *W. saturnus* var. *saturnus* and *W. saturnus* var. *suaveolens* is based on the following assimilation reactions on maltose and L-rhamnose: var. *saturnus*, maltose –/L-rhamnose + or maltose +/L-rhamnose v; var. *suaveolens*, maltose –/L-rhamnose –.

7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is white to grayish-white, moderately glistening, and butyrous. Colony margins are finely lobed.

Formation of ascospores: Conjugation occurs between cells and their buds, between independent cells, or rarely asci are unconjugated. Asci contain one to four elongated saturn-shaped ascospores which become moderately refractile upon release from their deliquescent asci (Fig. 190). Single-ascospore isolates from four-spored asci form sporogenous colonies, thus suggesting the species to be homothallic.

Ascospores were observed on 5% malt extract agar after 2 weeks at 25°C.

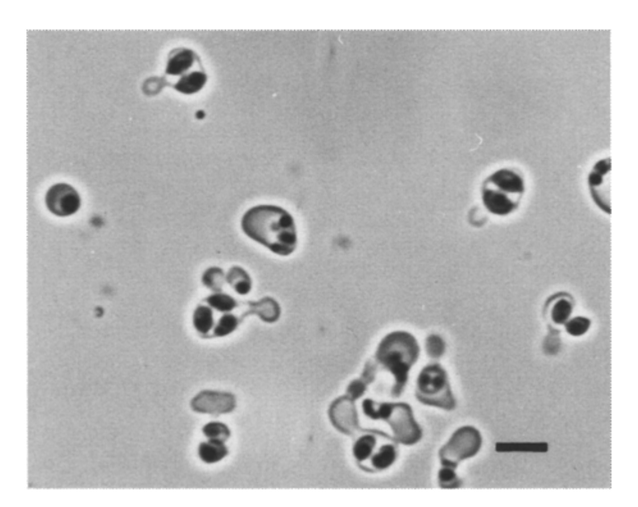


Fig. 190. *W. californica*, CBS 5760. Asci with ascospores, after 4 days on 5% malt extract agar at 25°C. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Starch formation	–
5-Keto-D-gluconate	v	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 7 (Yamada et al. 1973a).
Mol% G+C: 43.9, 44.1, two strains (T_m: Nakase and Komagata 1971b); 42.6–43.3, type and four additional strains (BD: Kurtzman 1991a).

Origin of the strains studied: This species has a worldwide distribution: soil, Australia (2), Belgium (1), Canada (1), India (1), New Zealand (4), U.S.A. (13); streams and lakes, U.S.A. (2); frass from the momi fir

(*Abies firma* Sieb & Zucc.), Japan (1), European beech (*Fagus silvaticus* L.), Sweden (1), mountain ash (*Sorbus americana* L.), U.S.A. (1), elm (*Ulmus* sp.), U.S.A. (1); flux of the koa acacia (*Acacia koa* Gray), U.S.A. (1); shrimp, U.S.A. (1); bear (*Ursus actos yesoensis*) dung, Japan (2); ox dung, France (1); unknown (2).

Type strain: CBS 252 (NRRL Y-17395), isolated by F.M. Muller in California, U.S.A., from an enrichment culture of *Azotobacter*. The original description of the species was based on this strain. CBS 5760 (NRRL Y-1680), isolated from soil, Murray Bridge, South Australia, was considered by Wickerham (1951) as the most typical strain of the species and came to be regarded by some as the type strain.

Comments: The taxa designated *Hansenula dimennae, Pichia saturnospora* and *Endomycopsis fukushimae* were reported by Kurtzman (1991a) to show 94–100% nuclear DNA relatedness with *W. californica* and, therefore, represent synonyms of this latter species. *H. dimennae* had been separated from *W. californica* because of its failure to assimilate sucrose.

54.2. *Williopsis mucosa* (Wickerham & Kurtzman) Kurtzman (1991a)

Synonyms:
Pichia mucosa Wickerham & Kurtzman (1971)
Waltiozyma mucosa (Wickerham & Kurtzman) Muller & Kock (1986)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to mostly ellipsoidal, (2.5–5.1)×(4.5–8.0) µm, and occur singly or in pairs. Growth is tannish-white in color and mucoid.

Growth on the surface of assimilation media: Dry climbing pellicles are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows pseudohyphae to be common and highly branched. True hyphae are not present. Aerobic growth is raised, highly glistening, mucoid, gray-white in color and with an entire margin.

Formation of ascospores: Asci are unconjugated and persistent and each contains one or two spores. The spores are spheroidal to occasionally slightly ellipsoidal and have a thick equatorial ring. Colonies from single-ascospore isolates are sporogenous, but since four-spored asci were not available for testing, it is not certain that this species is homothallic.

Ascospores were observed on 5% malt extract- and YM agars after 1 week at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+/w
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	v		

Co-Q: 7 (Y. Yamada, personal communication).
Mol% G+C: 33.7, 34.3, two strains (BD: Kurtzman 1991a).

Origin of the strains studied: Soil, South Africa (1), U.S.A. (1).

Type strain: CBS 6341 (NRRL YB-1344), from soil, Illinois, U.S.A.

Comments: As discussed under Comments on the genus, *W. mucosa* was transferred to *Williopsis* from *Pichia* because of the overall similarity of its rRNA nucleotide sequences to other members of the genus (Liu and Kurtzman 1991). *W. mucosa* is unique among the saturn-spored yeasts because it produces substantial amounts of an extracellular polysaccharide. The polysaccharide is neutral and the only sugar present is D-mannose (Seymour et al. 1976).

W. mucosa was assigned to the newly described genus *Waltiozyma* by Muller and Kock (1986) on the basis of apparent uniqueness in cellular fatty acid composition and because it was believed to produce coenzyme Q-6 (Billon-Grand 1985), in contrast to Q-7 that is produced by other *Williopsis* spp., as well as by *Saturnispora* spp. Y. Yamada (personal communication) reexamined *W. mucosa* and reported that both known strains are characterized by coenzyme Q-7.

54.3. *Williopsis pratensis* Bab'eva & Reshetova (1979)

Synonym:
Komagataea pratensis (Bab'eva & Reshetova) Y. Yamada, Matsuda, Maeda, Sakakibara & Mikata (1994b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to short ellipsoidal, (2.8–6.0)×(3.0–7.3) µm, and single, in pairs, or in small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed but thin films and rings may occur.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae. Aerobic growth is white, dull-glistening, finely striated and butyrous. Colony margins are irregular.

Formation of ascospores: Ascospores were not observed in the present study. Bab'eva and Reshetova (1979) reported the formation of unconjugated, persistent asci having one or two spheroidal spores with ledges.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	w	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 39.9, CBS 7079 (BD: Kurtzman 1991a).

Origin of the strains studied: Soil from a meadow, Russia (2).

Type strain: CBS 7079 (NRRL Y-12696), from meadow soil, Russia.

54.4. *Williopsis salicorniae* Hinzelin, Kurtzman & M.Th. Smith (1991)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (3.0–6.5)×(3.0–8.0) μm, and single, in pairs, or in small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows neither pseudohyphae nor true hyphae, but "tree-like" outgrowths of undifferentiated cells occur infrequently. Aerobic growth is tannish-white, dull-glistening, and butyrous. Colony margins are irregular.

Formation of ascospores: Asci are unconjugated, persistent, and form one to four globose to subglobose spores having a thin equatorial ledge.

Ascospores were observed on 5% malt extract agar after 3–4 weeks at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	s
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	s	Citrate	–
D-Arabinose	s	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 36.7, CBS 8071 (BD: Hinzelin et al. 1991).

Origin of the strain studied: Brackish water, France.

Type strain: CBS 8071 (NRRL Y-12834), isolated from the brackish water of a salt spring near Château-Salins, France.

54.5. *Williopsis saturnus* (Klöcker) Zender (1925b)

This species has five varieties:

Williopsis saturnus (Klöcker) Zender var. *saturnus* (1991)

Synonyms:
Saccharomyces saturnus Klöcker (1902)
Willia saturnus (Klöcker) Hansen (1904)
Hansenula saturnus (Klöcker) H. & P. Sydow (1919)
Hansenula beijerinckii van der Walt (1957)
Williopsis beijerinckii (van der Walt) Naumov & Vustin (Naumov et al. 1981)
Hansenula coprophila Soneda (1959)

Williopsis saturnus **var.** ***mrakii*** **(Wickerham) Kurtzman (1991a)**

Synonyms:

Hansenula mrakii Wickerham (1951)

Williopsis mrakii (Wickerham) Naumov & Vustin (Naumov et al. 1981)

Williopsis saturnus **var.** ***sargentensis*** **(Wickerham & Kurtzman) Kurtzman (1991a)**

Synonyms:

Pichia sargentensis Wickerham & Kurtzman (1971)

Williopsis sargentensis (Wickerham & Kurtzman) Naumov (1987a)

Williopsis saturnus **var.** ***suaveolens*** **(Klöcker) Kurtzman (1991a)**

Synonyms:

Pichia suaveolens Klöcker (1912a)

Hansenula suaveolens (Klöcker) Dekker (Stelling-Dekker 1931)

Williopsis suaveolens (Klöcker) Naumov, Vustin & Bab'eva (Naumov et al. 1985a)

Williopsis saturnus **var.** ***subsufficiens*** **(Wickerham) Kurtzman (1991a)**

Synonyms:

Hansenula saturnus (Klöcker) H. & P. Sydow var. *subsufficiens* Wickerham (1969b)

Williopsis subsufficiens (Wickerham) Vustin, Naumov & Bab'eva (Vustin et al. 1982)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal, or occasionally elongate, (2.5–5.2)×(3.5–8.5) µm, and occur singly, in pairs, or small clusters. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles vary from thin and smooth to thick and folded, and may reach a height of 20 mm above the surface of the medium.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows only occasional primitive pseudohyphae. True hyphae are not produced. Aerobic growth is tannish-white, smooth, dull, and butyrous. Colony margins may be entire or finely lobed.

Formation of ascospores: Asci are generally unconjugated but occasionally conjugation between independent cells is observed. One to four saturn-shaped ascospores are formed per ascus (Fig. 191). The thickness of the rings on the spores varies, and spores from one strain may even lack rings (Wickerham 1970a). Asci are persistent. Single-spore isolates form sporogenous colonies indicating the species to be homothallic.

Ascospores were observed on 5% malt extract agar and on YM agar after 2 weeks at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+/w
Sucrose	+	Trehalose	–
Maltose	–		

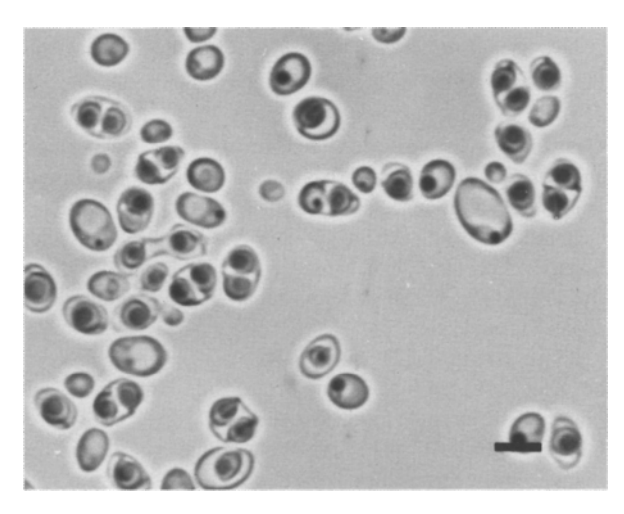


Fig. 191. *W. saturnus* var. *saturnus*, CBS 5761. Asci with ascospores after 4 days on YM agar at 25°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	v
Inulin	v	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	w/–
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: 7, varieties *saturnus, subsufficiens, mrakii* (Yamada et al. 1973a).

Mol% G + C: variety *saturnus*, 42.4–43.7, three strains (T_m), 44.3–44.6, five strains (BD); variety *mrakii*, 42.0, one strain (T_m), 44.3, two strains (BD); variety *sargentensis*, 44.1, one strain (BD); variety *suaveolens*, 44.5, 44.7, two strains (BD); variety *subsufficiens*, 42.9, 43.2, two strains (T_m), 43.5, 43.7, two strains (BD) (T_m: Nakase and Komagata 1971b; BD: Kurtzman 1991a).

Supplementary description of *W. saturnus* varieties: Variety *mrakii*: sucrose is neither fermented nor assimilated.

Variety *sargentensis*: sucrose is neither fermented nor assimilated, nitrate is not assimilated, an exogenous supply of vitamins is required for growth.

Variety *suaveolens*: neither maltose nor L-rhamnose are assimilated, whereas for the variety *saturnus*, one or both of these sugars are assimilated.

Variety *subsufficiens*: sucrose is fermented and assimilated but an exogenous supply of vitamins is required for growth.

Origin of the strains belonging to the variety *saturnus*: The taxon has been isolated worldwide from the following: soil, Egypt (1), Liberia (6), New Zealand (1), South Africa (2), U.S.A. (21); streams, lakes, bogs, U.S.A. (10); leaf litter, Japan (1), U.S.A. (1); tree frass, U.S.A. (3); vegetables, Japan (1); elephant dung, Japan (1); unknown (7).

Type strain: CBS 254 (NRRL Y-17396), believed to be Klöcker's original isolate from soil, Himalayas, and obtained through Winge. Wickerham (1951) considered CBS 5761 (NRRL Y-1304) to be a typical strain, and this inadvertently came to be regarded as the type strain.

Origin of the strains belonging to the variety *mrakii*: Soil, India (1), New Guinea (1); swamp water, Wyoming, U.S.A. (1); water from a hot spring, California, U.S.A. (1).

Type strain: CBS 1707 (NRRL Y-1364), isolated from soil in New Guinea.

Origin of the strain belonging to the variety *sargentensis*: Outlet of a small lake at Camp Sargent near Peterborough, New Hampshire, U.S.A.

Type strain: CBS 6342 (NRRL YB-4139).

Origin of the strains belonging to the variety *suaveolens*: Soil, Canada (1), Denmark (1), New Zealand (2), U.S.A. (3); ponds and streams, U.S.A. (6); unknown (2).

Type strain: CBS 255 (NRRL Y-17391), Klöcker's culture from soil, Denmark.

Origin of the strains belonging to the variety *subsufficiens*: Soil, Liberia (2), Illinois, U.S.A. (1).

Type strain: CBS 5763 (NRRL YB-1657), from soil, Liberia.

Comments: *W. saturnus* and the four taxa designated as its varieties in this treatment have had a long and varied taxonomic history. Wickerham (1969a) attempted to address the issue of species delimitation in the *W. saturnus* complex by measuring the fertility of intertaxon pairings. Hybrids were obtained between the following pairs: *Hansenula californica* and *H. dimennae*; *H. mrakii* and *H. beijerinckii*; *H. saturnus* var. *saturnus* and *H. beijerinckii*; and *H. saturnus* var. *subsufficiens* and *H. beijerinckii.* Hybrids showed reduced sporulation and a greater proportion of poorly formed ascospores, but the analysis was complicated by the finding that hybrids failed to form in certain intraspecific pairings.

Naumov and colleagues (Naumov et al. 1985a, Naumov 1987a) extended Wickerham's genetic studies by using auxotrophic mutants, which allowed hybridizations to be followed more accurately, and by analysis of any resulting single-spore isolates. Crosses of the species pairs *W. saturnus* var. *saturnus* × *W. beijerinckii*, *W. saturnus* var. *saturnus* × *W. mrakii* and *W. beijerinckii* × *W. mrakii* yielded hybrids producing ascospores with only 0–4% viability, leading to the interpretation that all four taxa represent biologically separate sibling species (Naumov 1987a). However, in earlier work, Naumov et al. (1985a) suggested that *W. (Zygowilliopsis) californica* and its suspected synonyms *Endomycopsis fukushimae* and *Pichia saturnospora* were conspecific on the basis of crosses that yielded progeny viabilities much like those found among the *W. saturnus* complex.

Kurtzman (1991a) addressed the taxonomy of the *W. saturnus* complex from the perspective of nuclear DNA relatedness. DNA complementarity ranged from 36–72%. Thus, as with the foregoing genetic crosses, the extent of DNA relatedness approaches that of separate species in some cases, but is nonetheless, greater than previously seen among many closely related sibling species (Kurtzman 1987b). Because of the uncertainties expressed about genetic isolation among the strains, all taxa showing intermediate DNA relatedness have been defined as varieties of *W. saturnus.* This approach also serves to emphasize their close genetic relatedness in a way not possible if some of the taxa are designated as separate species. Consistent with this treatment is the similarity of rRNA nucleotide sequences exhibited by members of the group (Liu and Kurtzman 1991).

W. saturnus var. *sargentensis* was earlier classified as *Pichia sargentensis* (Wickerham and Kurtzman 1971). The relatively high DNA relatedness (68%) detected between this taxon and *W. saturnus* var. *mrakii* (Kurtzman 1991a) further argues that ability to assimilate nitrate as a sole source of nitrogen offers little taxonomic resolution, an observation in keeping with other work (Kurtzman 1984a).

Comments on the genus

The genus *Williopsis,* as defined by Zender (1925b), was based mainly on the formation of saturn-shaped ascospores by the species assigned to it rather than on ability to assimilate nitrate as a sole source of nitrogen as later became the basis for separating *Hansenula* and *Pichia* (Stelling-Dekker 1931). More recent taxonomic treatments have discarded use of *Williopsis* (Wickerham 1951, Lodder and Kreger-van Rij 1952, Lodder 1970, Kreger-van Rij 1984a) in favor of assigning nitrate-assimilating species to *Hansenula* and placing in *Pichia* those species that do not assimilate nitrate. Von Arx et al. (1977) revived the use of *Williopsis* but seem to have restricted species assignment to those saturn-spored taxa that were formerly placed in *Hansenula.*

Naumov (1987a) has argued for retention of *W. californica* in *Zygowilliopsis* as proposed earlier when the genus was described by Kudryavtsev (1960). Wickerham (1951) examined the species now assigned to *Williopsis* and pointed out that all form some haploid cells, thus negating the distinction between haploid and diploid species, the primary criterion for separation of *Zygowilliopsis* and *Williopsis.*

Kurtzman (1991a) measured the extent of nuclear DNA complementarity among saturn-spored taxa assigned to *Williopsis* and *Pichia* thus defining species. Because the resolution of this methodology extends only to definition of closely related sibling species (Kurtzman 1987b), Liu and Kurtzman (1991) estimated species relationships from rRNA divergence. Results from these comparisons were rather surprising. The saturn-spored *Pichia* spp. formed a close cluster that was well-separated from *Williopsis,* but all five *Williopsis* spp. were rather widely separated from one another. Because of their genetic isolation, the four *Pichia* spp. were assigned to the newly described genus *Saturnispora* (Liu and Kurtzman 1991). The apparent great phylogenetic distance between species of *Williopsis* raised several possibilities: the taxa represented five monotypic sibling genera, rates of nucleotide substitution were abnormally high causing the genetic distance to appear greater than it is, or that there are numerous taxa missing from the clade. In view of the uncertainty, Liu and Kurtzman (1991) considered the five species to be members of *Williopsis.*

Kurtzman and Robnett (unpublished data) reexamined the phylogeny of *Williopsis* by including the species in rRNA sequence analyses of *Pichia.* The genus *Pichia* separated into six major clades with *Williopsis* species distributed among several of them. These data demonstrated *Williopsis* and *Pichia* to be polyphyletic and therefore artificial genera. Additional work is required before the species can be reassigned to genera that are phylogenetically circumscribed.

At present, *Williopsis* and *Saturnispora* can be separated on standard growth tests because all species assigned to *Williopsis* assimilate D-xylose whereas *Saturnispora* species do not. D-xylose assimilation is regarded as a fortuitous phenotypic marker and not of phylogenetic significance.

55. *Yarrowia* van der Walt & von Arx

C.P. Kurtzman

Diagnosis of the genus

Asexual reproduction is by multilateral budding on a narrow base. Arthroconidia may occasionally be formed as well. Budded cells are spheroidal, ellipsoidal, or often elongate. True hyphae and pseudohyphae are formed; true hyphae have septa with a single central micropore.

Asci are unconjugated and generally arise from diploid hyphae. The asci, which slowly deliquesce, form 1–4 ascospores that may be spheroidal, hat-shaped, hemispheroidal, or somewhat angular.

Sugars are not fermented. Nitrate is not assimilated. Lipases, proteases, and urease are produced. Coenzyme Q-9 is formed. Diazonium blue B reaction is negative.

Type species

Yarrowia lipolytica (Wickerham, Kurtzman & Herman) van der Walt & von Arx

Species accepted

1. *Yarrowia lipolytica* (Wickerham, Kurtzman & Herman) van der Walt & von Arx (1980)

Systematic discussion of the species

55.1. *Yarrowia lipolytica* (Wickerham, Kurtzman & Herman) van der Walt & von Arx (1980)

Anamorph: *Candida lipolytica* (F.C. Harrison) Diddens & Lodder
Synonyms:
Endomycopsis lipolytica Wickerham, Kurtzman & Herman (1970b)
Saccharomycopsis lipolytica (Wickerham, Kurtzman & Herman) Yarrow (1972)
Mycotorula lipolytica F.C. Harrison (1928)
Torula lipolytica (F.C. Harrison) Jacobsen (Lodder 1934)
Candida lipolytica (F.C. Harrison) Diddens & Lodder (1942)
Azymoprocandida lipolytica (F.C. Harrison) Novák & Zsolt (1961)
Monilia cornealis Nannizzi (Bencini and Federici 1928)
Proteomyces cornealis (Nannizzi) Dodge (1935)
Pseudomonilia deformans Zach (Wolfram and Zach 1934b)
Candida deformans (Zach) Langeron & Guerra (1938)
Candida lipolytica (F.C. Harrison) Diddens & Lodder var. *deformans* (Zach) van Uden & H.R. Buckley (1970)
Candida olea van Rij & Verona (1949)
Candida paralipolytica K. Yamada & Ota (1963)
Candida lipolytica (F.C. Harrison) Diddens & Lodder var. *thermotolerans* Blagodatskaya & Kocková-Kratochvílová (1973)
Candida oleophila Iizuka, Shimizu, Ishii & Nakajima (1967) nom. nud.
Torulopsis petrophilum Takeda, Iguchi, Tsuzuki & Nakano (1972) nom. nud.
Candida petrophilum Takeda, Iguchi, Tsuzuki & Nakano (1972) nom. nud.
Candida pseudolipolytica Blagodatskaya & Kocková-Kratochvílová (1973)
Saccharomycopsis pseudolipolytica Blagodatskaya (1979)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal, ellipsoidal to elongate, (3.0–5.0)×(3.3–15.0) μm, and single, in pairs or small clusters. Growth is butyrous to mycelial and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are formed and may be thin or rather thick.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows pseudohyphae, and true hyphae are usually present as well. The septa of true hyphae have a single central pore that lacks tapered edges (Kreger-van Rij and Veenhuis 1973). Aerobic growth is white to tannish-white and smooth-glistening to dull and convoluted. Colony margins may be entire or lobed.

Formation of ascospores: This species is heterothallic. Asci are unconjugated and may form from natural diploids or from diploids resulting from conjugation of complementary mating types. Asci are usually produced on hyphal cells but infrequently an ascus will arise on a single blastoconidium. Asci may be either stalked or sessile, and they become deliquescent as they mature. Generally, one to four ascospores are formed in each ascus (Fig. 192). Ascospore shape is quite variable and is determined in part by the mating types paired. Ascospores from NRRL YB-

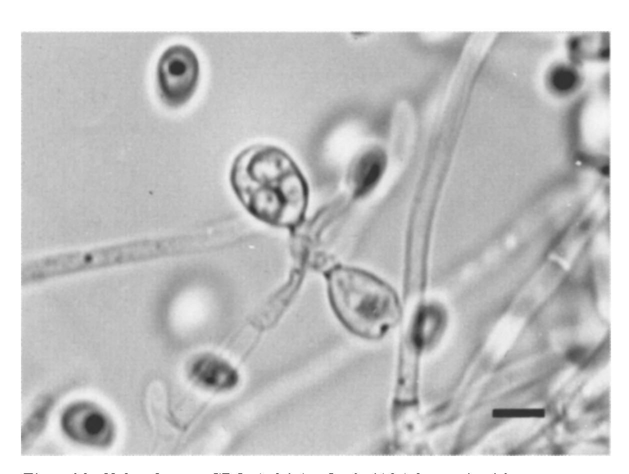


Fig. 192. *Y. lipolytica*, CBS 6124.1×CBS 6124.2. Asci with ascospores, after 1 week at 25°C, on YM agar. Bar = 5 μm.

423-3×NRRL YB-423-12 are spheroidal, hat-shaped, or irregular, and may be roughened, whereas ascospores from NRRL YB-421×NRRL YB-423-12 are hemispheroidal and somewhat saucer-like.

Ascospores were observed on YM agar after 3–7 days at 25°C. Herman (1971b) reported increased fertility and ascosporulation when complementary mating types were paired on RG medium.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	w/–	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	w/–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	+
Saccharate	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: 9 (Yamada and Kondo 1972b).

Mol% G+C: 49.6, CBS 599 (T_m: Stenderup and Bak 1968); 50.2, CBS 6124 (T_m: Nakase and Komagata 1971f).

Origin of the strains studied: Refrigerated meat products, U.S.A. (8); *Pseudomonilia deformans* (CBS 2071, NRRL Y-321); *Monilia cornealis* (CBS 2070, NRRL Y-323), from the cornea of a human eye; petroleum products (7); agricultural (corn) processing plant, Illinois, U.S.A. (17); compost soil, Hungary (1); unknown (17).

Complementary mating types: NRRL YB-423-3 (CBS 6124.1) and NRRL YB-423-12 (CBS 6124.2), single-ascospore isolates from NRRL YB-423.

Type strain: CBS 6124 (NRRL YB-423), an ascosporogenous strain isolated by Wickerham from corn fiber tailings, Illinois, U.S.A.

Comments on the genus

Wickerham et al. (1970a,b) discovered the teleomorph of *Candida lipolytica*, which they described as *Endomycopsis lipolytica*. However, van der Walt and Scott (1971b) pointed out that *Endomycopsis* was an obligate synonym of *Saccharomycopsis* Schiönning, but they placed only some of the species in other genera. Later, Yarrow (1972) transferred *E. lipolytica* to the genus *Saccharomycopsis*. Relative to other members of *Saccharomycopsis*, *S. lipolytica* appeared unique because of the shape of its ascospores and the presence of coenzyme Q-9. Based on these criteria, van der Walt and von Arx (1980) described the genus *Yarrowia* for placement of *S. lipolytica*.

Through comparisons of 18S rRNA sequences, Barns et al. (1991) demonstrated *Y. lipolytica* to be phylogenetically distant from selected members of *Candida* and several teleomorphic ascomycetous yeast genera. Kurtzman and Robnett (1994a, 1995) showed from partial rRNA/rDNA sequence analyses that *Y. lipolytica* is only distantly related to most other known ascomycetous yeasts and that its nearest neighbor appears to be *Wickerhamiella domercqiae*.

Y. lipolytica has several physiological properties of industrial significance. The species is well-known for production of proteases, lipase, and utilization of *n*-paraffins (Peters and Nelson 1948, Tsugawa et al. 1969). This last property focused interest on *Y. lipolytica* as a potential source of yeast protein from hydrocarbons, a popular idea in an era of low cost petroleum. The discovery in the late 1960's that high yields of citric acid were produced by *Y. lipolytica* (Yamada 1977) signaled a dramatic change in the fermentation industry, because the yields of this industrially important acidulant were about twice that obtained from *Aspergillus niger*, the industry mainstay since the early 1900's.

56. *Zygoascus* M.Th. Smith

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is by multilateral budding, and formation of pseudomycelium and true hyphae with blastoconidia. Hyphal septa have micropores.

Asci are formed after fusion of opposing hyphal cells and are obovate to spheroidal, persistent and contain 1–4 hemispheroidal to galeate ascospores.

Sugars are fermented. Nitrate is not assimilated. Diazonium blue B reaction is negative.

Type species

Zygoascus hellenicus M.Th. Smith

Species accepted

1. *Zygoascus hellenicus* M.Th. Smith (1986)

Systematic discussion of the species

56.1. *Zygoascus hellenicus* M.Th. Smith (1986)

Anamorph: *Candida hellenica* (Verona & Picci) D.S. King & S.-C. Jong

Synonyms:
Trichosporon hellenicum Verona & Picci (1958)
Candida hellenica (Verona & Picci) D.S. King & S.-C. Jong (1977)
Candida steatolytica Yarrow (1969)
Candida inositophila Nakase (1975)
Pichia hangzhouana Lu & M.X. Li (1989)

Growth on glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal, long ovoidal, sausage-shaped, or elongate to cylindroidal, (2.0–5.0)×(4.5–15.5) μm. Pseudomycelium with long branches and mycelial cells (up to 30 μm in length) may also be present. A sediment and ring with islets or a thick wrinkled pellicle are formed.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is white or cream-colored, shiny or dull, soft or slightly membranous, and smooth or delicately wrinkled. A mycelial border is present.

Dalmau plate culture on corn meal agar: Both pseudomycelium and true hyphae are abundantly formed. Pseudohyphal cells slender and often curved. Ovoidal blastoconidia are present.

Formation of ascospores: The species is heterothallic. Asci, which may be single or in clusters, form either directly from conjugation of opposite hyphal cells, where the conjugation bridge forms one or more lateral outgrowths which develop into asci, or from apical cells which arise after sequential buddings of the lateral outgrowths. Asci are obovoidal to spheroidal and persistent. One to four hemispheroidal to hat-shaped ascospores are formed (Fig. 193).

Ascospores were observed on 5% Difco malt agar within 7–21 days at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	v
Sucrose	+	Trehalose	+
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	v
L-Arabinose	+	Citrate	v
D-Arabinose	v	Inositol	+
D-Ribose	v	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

50% Glucose	+	Growth at 37°C	+
60% Glucose	–	Growth at 42°C	–
Fat splitting	v		

Co-Q: 9 (Yamada et al. 1976b).

Mol% G+C: 43.9, CBS 6736 (T_m: Nakase 1975); 43.7, CBS 4099 (T_m: Meyer et al. 1984); 43.5–44.9, 3 strains, CBS 5839, CBS 6726, CBS 7115 (T_m: Smith 1986).

Origin of the strains studied: CBS 5839, mastitic bovine udder, van der Walt, mating type α, type strain of *Candida steatolytica*; CBS 6736, washings of ion exchange resin, Nakase, mating type *a*, type strain of *Candida inositophila*; CBS 4099, fermenting grape must, Verona, mating type *a*, type strain of *Trichosporon hellenicum*; mating type *a* from grape must (1), organic

Table 45
Differentiating characteristics of some genera of the Endomycetaceae and Saccharomycetaceae (classification after von Arx 1981)

Family/genus	Structure of septa	Apical cell on asci	Deliquescence of asci	Coenzyme Q system	Urease
Endomycetaceae					
Ambroziozyma	Dolipore + plug	–	v	7	–
Hormoascus	Dolipore + plug	–	+	7	–
Botryoascus	Micropores	–	+	8	–
Endomyces	Micropores	–	+	8	–
Hyphopichia	Micropore (1 central)	–	+	8	–
Stephanoascus	Micropore(s)	+	–	9	–
Zygoascus	Micropore (1 central)	–	–	9	–
Saccharomycetaceae					
Yarrowia	Micropore (1 central)	–	+	9	+
Saccharomycopsis	Micropores	–	+	8	–

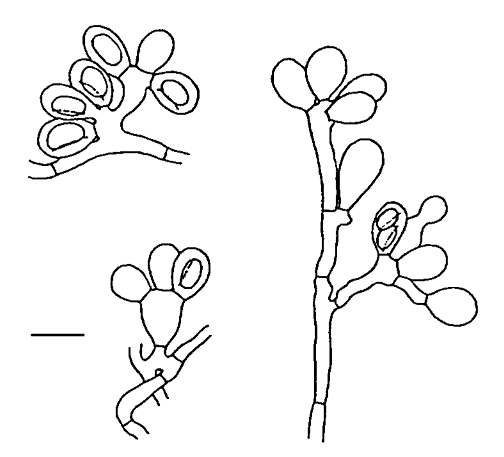


Fig. 193. *Z. hellenicus*, CBS 5839×CBS 6736. Asci with ascospores on 5% Difco malt agar after 7 days at 25°C. Bar = 10 μm.

acid fermentation (1), tunnels of *Xyleborus ferrugineus* in *Harpephyllum caffrum* (1), synthetic medium of sucrose and ammonium, pH 1.5–3.5 (1), unknown (1), and mating type α from equipment in soft drink factory (1), deep-frozen orange juice (1).

Complementary mating types: CBS 5839 and CBS 6736.

Type strain: Holotype as dried sporulating specimen of CBS 5839×CBS 6736. Living strains CBS 5839 and CBS 6736.

Comments on the genus

The genus *Zygoascus* is characterized by the production of budding cells, pseudohyphae, septate hyphae and asci with 1–4 hemispheroidal to galeate ascospores which have a distinct electron-dense base (Smith 1986). In this respect, *Zygoascus* resembles eight yeast genera as classified by von Arx (1981) in the Endomycetaceae and Saccharomycetaceae. However, *Zygoascus* can be distinguished from these genera either by morphological differences of the septa and the asci or by differences in biochemical properties (Table 45). Kurtzman and Robnett (unpublished data) found *Pichia hangzhouana* and *Z. hellenicus* to have identical nucleotide sequences in the 5′ end of the large subunit rDNA, indicating that the two taxa are conspecific.

57. *Zygosaccharomyces* Barker

C.P. Kurtzman

Diagnosis of the genus

Vegetative reproduction is by multilateral budding. Cells are spheroidal, ellipsoidal or elongate. Pseudohyphae may be present but true hyphae are not formed.

Asci are persistent and may be unconjugated or show conjugation between a cell and its bud; most frequently, asci form following conjugation between independent cells, often with both cells producing ascospores. Ascospores are smooth, spheroidal to ellipsoidal, with 1–4 per ascus.

Glucose is fermented. Nitrate is not assimilated. Pellicles are not formed on liquid media. Coenzyme Q-6 is produced. Diazonium blue B reaction is negative.

Type species

Zygosaccharomyces barkeri Saccardo & Sydow

An original culture no longer exists, but *Z. barkeri,* the type species of *Zygosaccharomyces,* may be a synonym of *Z. rouxii*.

Species accepted

1. *Zygosaccharomyces bailii* (Lindner) Guilliermond (1912)
2. *Zygosaccharomyces bisporus* H. Naganishi (1917)
3. *Zygosaccharomyces cidri* (Legakis) Yarrow (1977)
4. *Zygosaccharomyces fermentati* H. Naganishi (1928)
5. *Zygosaccharomyces florentinus* Castelli ex Kudryavtsev (1960)
6. *Zygosaccharomyces mellis* Fabian & Quinet (1928)
7. *Zygosaccharomyces microellipsoides* (Osterwalder) Yarrow (1977)
8. *Zygosaccharomyces mrakii* Capriotti (1958)
9. *Zygosaccharomyces rouxii* (Boutroux) Yarrow (1977)

Key to species

See Table 46.

1.	a	Growth with 0.1% cycloheximide → 2		
	b	Absence of growth with 0.1% cycloheximide → 5		
2(1).	a	Growth at 37°C	*Z. fermentati*:	p. 427
	b	Absence of growth at 37°C → 3		
3(2).	a	Inositol required for growth	*Z. cidri*:	p. 426
	b	Inositol not required for growth → 4		
4(3).	a	α-Methyl-D-glucoside assimilated	*Z. florentinus*:	p. 428
	b	α-Methyl-D-glucoside not assimilated	*Z. mrakii*:	p. 430
5(1).	a	Melibiose assimilated	*Z. microellipsoides*:	p. 430
	b	Melibiose not assimilated → 6		
6(5).	a	Growth on agar with 1% acetic acid → 7		
	b	Absence of growth on agar with 1% acetic acid → 8		
7(6).	a	Trehalose assimilated	*Z. bailii*:	p. 424
	b	Trehalose not assimilated	*Z. bisporus*:	p. 426
8(6).	a	Growth with 16% NaCl/5% glucose	*Z. rouxii*:	p. 431
	b	Absence of growth with 16% NaCl/5% glucose	*Z. mellis*:	p. 429

Systematic discussion of the species

57.1. *Zygosaccharomyces bailii* (Lindner) Guilliermond (1912)

Synonyms:

Saccharomyces bailii Lindner (1895)
Zygosaccharomyces mandshuricus Saito (1914)
Zygosaccharomyces nishiwakii Guilliermond (1930)
Zygosaccharomyces naniwaensis Otani (1931)
Zygosaccharomyces sake Suminoe (Naganishi 1941)
Saccharomycodes mestris Marcilla & Feduchy (1943)
Zygosaccharomyces acidifaciens Nickerson (1943)
Saccharomyces acidifaciens (Nickerson) Lodder & Kreger-van Rij (1952)
Saccharomyces elegans Lodder & Kreger-van Rij (1952)
Saccharomyces elegans Lodder & Kreger-van Rij var. *intermedia* Verona & Zardetto de Toledo (1959b) nom. nud.

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (3.1–7.0)×(3.6–11.8) μm, and single or in pairs. Growth is butyrous and tannish-white in color.

Table 46
Key characters of species in the genus *Zygosaccharomyces*

Species	Assimilation			Growth				
	Tre	Mel	α-MG	0.1% Cycloheximide	W/o inositol	GTYE/1% acetic acid	16%NaCl/ 5%glucose	37°C
Zygosaccharomyces bailii	×	–	–	–	+	×	×	v
Z. bisporus	–	–	–	–	+	×	v	–
Z. cidri	+	+	+	+	–	–	–	–
Z. fermentati	+	v	+	+	–	–	v	+
Z. florentinus	+	+	+	+	+	–	–	–
Z. mellis	v	–	–	–	+	–	–	–
Z. microellipsoides	+	+	–	–	+	–	–	–
Z. mrakii	–	+	–	+	+	–	–	–
Z. rouxii	×	–	–	–	+	–	+	v

[a] Abbreviations: Tre, trehalose; Mel, melibiose; α-MG, α-methyl-D-glucoside; GTYE/1% acetic acid, glucose-tryptone-yeast extract agar with 1% acetic acid; 16%NaCl/5%glucose, 16%NaCl/5%glucose in yeast nitrogen base.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass often shows pseudohyphal outgrowths, but the pseudohyphae usually show little branching and the cells are seldom elongated. True hyphae are not formed. Aerobic growth is tannish-white, dull-glistening with a smooth to finely convoluted surface, butyrous, and with margins that are entire to finely lobed. A faint, acidic odor is present.

Formation of ascospores: Asci are persistent and generally conjugated with each conjugant forming one or two smooth, globose to ovoidal ascospores (Fig. 194). The species appears homothallic.

Ascospores were observed on YM and 5% malt extract agars after 3–30 days at 25°C.

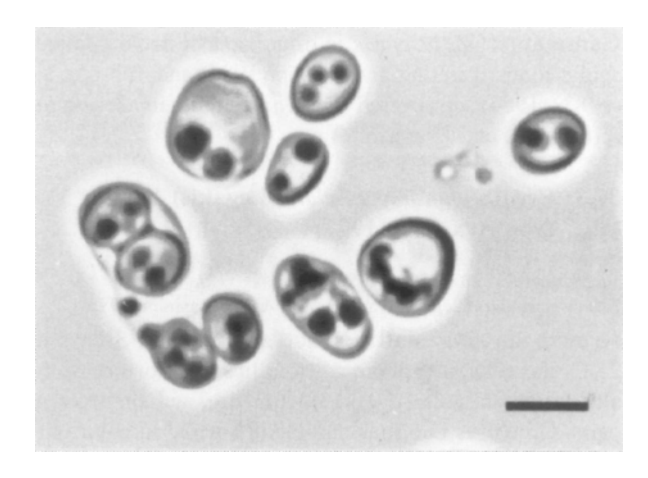


Fig. 194. *Z. bailii*, CBS 1097. Conjugated ascus with ascospores, after 3 weeks on 5% malt extract agar at 25°C. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	v	Trehalose	w/–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	v	Glycerol	+/w
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	+/w	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	18% NaCl/5% glucose	+/w
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Gelatin liquefaction	–
10% NaCl/5% glucose	+/w	GTYE/1% acetic acid[1]	+/w
12.5% NaCl/5% glucose	+/w	0.1% Cycloheximide	–
15% NaCl/5% glucose	+/w	w/o Inositol	+
16% NaCl/5% glucose	+/w	Growth at 37°C	v
17% NaCl/5% glucose	+/w	Growth at 40°C	n

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: 6, CBS 680 and 2 additional strains (Yamada et al. 1976b).

Mol% G + C: 40.5, CBS 680 (T_m: Nakase and Komagata 1971e); 43.8–45.5, CBS 680 and 10 additional strains (BD: Kurtzman 1990c).

Origin of the strains studied: CBS 680 (NRRL

Y-2227), Inst. of Brewing, Tokyo; apple juice, Netherlands (1); spoiled salad dressing, U.S.A. (9).

Type strain: CBS 680.

Comments: *Z. bailii* has a worldwide distribution and is often isolated from spoiled acidified condiments. Kurtzman et al. (1971) showed that much of the yeast spoilage of mayonnaise and salad dressing was due to *Z. bailii.*

Z. bailii is of potential industrial interest. Nickerson (1943), Nickerson and Carroll (1945) and Spencer and Sallans (1956) reported some strains to produce large amounts of L-arabitol, glycerol, erythritol and mannitol.

57.2. *Zygosaccharomyces bisporus* H. Naganishi (1917)

Synonym:

Saccharomyces bisporus (H. Naganishi) Lodder & Kreger-van Rij (1952) [nec *Zygosaccharomyces bisporus* Anderson (1917)]

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.3–5.2)×(2.4–10.1) µm, and single or in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass may occasionally show a few short chains of ellipsoidal cells, but neither well-developed pseudohyphae nor true hyphae are formed. Aerobic growth is butyrous, white to tannish-white, dull-glistening, with a smooth surface, and with margins that are entire or lobed. A faint, acidic odor is present.

Formation of ascospores: Asci are persistent and often conjugated, with each conjugant forming one or two smooth, globose to ovoidal ascospores (Fig. 195). The species appears homothallic.

Ascospores were observed on YM and 5% malt extract agars after 5–30 days at 25°C.

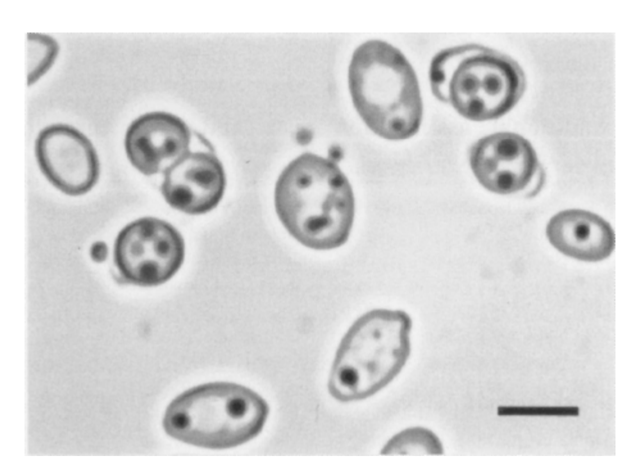


Fig. 195. *Z. bisporus*, NRRL Y-7253. Conjugated asci with ascospores after 20 days on 5% malt extract agar at 25°C. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	18% NaCl/5% glucose	v
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Gelatin liquefaction	–
10% NaCl/5% glucose	+	GTYE/1% acetic acid[1]	+/w
12.5% NaCl/5% glucose	v	0.1% Cycloheximide	–
15% NaCl/5% glucose	v	w/o Inositol	+
16% NaCl/5% glucose	v	Growth at 37°C	–
17% NaCl/5% glucose	v		

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: 6, CBS 702 and 1 additional strain (Yamada et al. 1976b).

Mol% G+C: 44.1, 43.7, CBS 702, IFO 0723 (T_m: Nakase and Komagata 1971e); 44.0, CBS 702 (BD: Price et al. 1978); 45.0–45.6, CBS 702 and 2 additional strains (BD: Kurtzman 1990c).

Origin of the strains studied: CBS 702 (NRRL Y-12626), from K. Saito; CBS 1082, from tea-beer fungus, Java; NRRL Y-7253, from spoiled salad dressing, U.S.A.

Type strain: CBS 702.

Comments: *Z. bisporus* is often isolated from spoiled, acidic foods. Painting and Kirsop (1984) and Toh-e et al. (1982, 1984) discovered DNA plasmids in *Z. bailii, Z. bisporus* and *Z. rouxii.*

57.3. *Zygosaccharomyces cidri* (Legakis) Yarrow (von Arx et al. 1977)

Synonyms:

Saccharomyces cidri Legakis (1961)

Torulaspora cidri (Legakis) van der Walt & E. Johannsen (1975a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, or occasionally cylindrical, (2.3–5.9)×(3.0–6.2) µm, and single or in pairs. Growth is butyrous and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass may occasionally show short chains of ellipsoidal cells, but neither well-developed pseudohyphae nor true hyphae are formed. Aerobic growth is butyrous, tannish-white, dull to glistening with a smooth surface, and with entire to finely lobed margins. A faint, acidic odor is present.

Formation of ascospores: Asci are persistent and generally conjugated, with each conjugant forming one, two, or three smooth, globose to ovoidal ascospores. (Fig. 196). The species appears homothallic.

Ascospores were observed on YM and 5% malt extract agars after 2–4 weeks at 25°C.

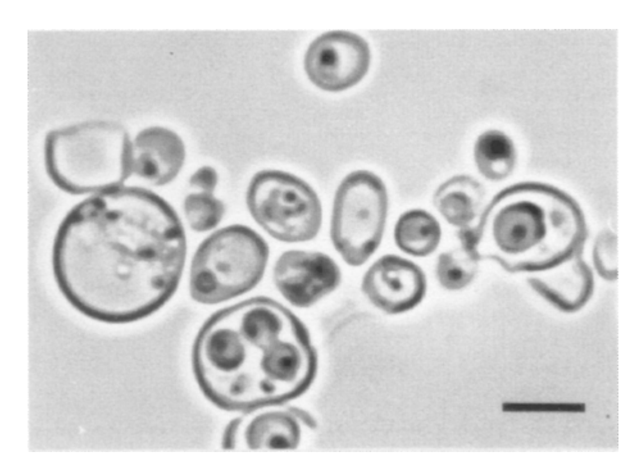


Fig. 196. *Z. cidri*, CBS 4575. Conjugated ascus with ascospores after 4 weeks on 5% malt extract agar at 25°C. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	–
Inulin	+	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	GTYE/1% acetic acid[1]	–
10% NaCl/5% glucose	+	0.1% Cycloheximide	+
12.5% NaCl/5% glucose	w/–	w/o Inositol	–
15% NaCl/5% glucose	–	Growth at 37°C	–

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: Not determined.

Mol% G + C: 42.6, CBS 4575 (BD: Price et al. 1978); 43.9, 43.3, CBS 4575, CBS 2950 (BD: Kurtzman 1990c).

Origin of the strains studies: CBS 4575 (NRRL Y-12634) and CBS 2950 (NRRL Y-12635), both from cider.

Type strain: CBS 4575.

57.4. *Zygosaccharomyces fermentati* H. Naganishi (1928)

Synonyms:

[non *Zygosaccharomyces fermentati* (Saito) Krumbholz (1933)]
Debaryomyces mandshuricus H. Naganishi (1924)
Zymodebaryomyces mandshuricus (H. Naganishi) Novák & Zsolt (1961)
Torulaspora manchurica (H. Naganishi) van der Walt & E. Johannsen (1975a)
Saccharomyces montanus Phaff, M.W. Miller & Shifrine (1956)
Saccharomyces malacitensis Santa María (1960) nom. nud.
Saccharomyces nilssoni (Capriotti) Santa María var. *malacitensis* Santa María (1963b)
Saccharomyces amurcae van der Walt (1970c)
Torulaspora amurcae (van der Walt) van der Walt & E. Johannsen (1975a)
Saccharomyces albasitensis Santa María (1978)
Saccharomyces astigiensis Santa María (1978)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to cylindrical, (2.0–7.1)×(3.0–8.1) µm, and occur singly, in pairs or in small clusters. Growth is butyrous and tannish-white.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass may show simple, branched pseudohyphae, but true hyphae are not present. Aerobic growth is butyrous, tannish-white, and with a smooth, glistening surface. Colony margins are entire to irregularly lobed. A faint, acidic, ester-like odor is present.

Formation of ascospores: Asci are persistent and may be unconjugated or show conjugation between independent cells. Asci form one to four spheroidal to ovoidal ascospores that appear smooth (Fig. 197). The species is probably homothallic.

Ascospores were observed on YM, corn meal and 5% malt extract agars after 2–4 weeks at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	v
Sucrose	+	Trehalose	+
Maltose	+		

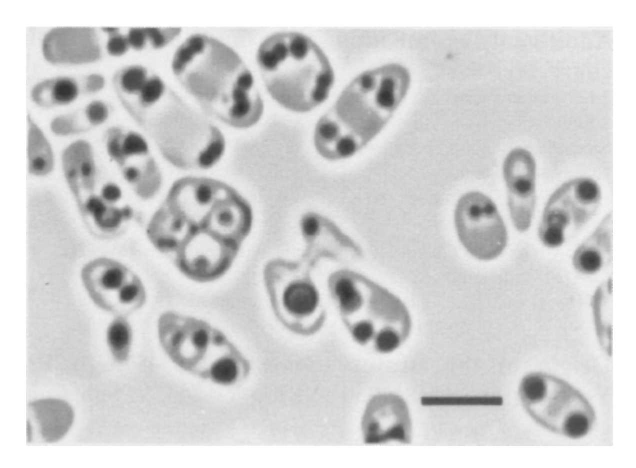


Fig. 197. *Z. fermentati*, CBS 707. Conjugated and unconjugated asci with ascospores after 4 weeks on 5% malt extract agar at 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	v	D-Gluconate	v
Soluble starch	–	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	18% NaCl/5% glucose	–
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Gelatin liquefaction	–
10% NaCl/5% glucose	v	GTYE/1% acetic acid[1]	–
12.5% NaCl/5% glucose	v	0.1% Cycloheximide	+
15% NaCl/5% glucose	v	w/o Inositol	–
16% NaCl/5% glucose	v	Growth at 37°C	+
17% NaCl/5% glucose	v		

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: 6, 2 strains (Yamada et al. 1976b).

Mol% G + C: 42.9, 43.4, 2 strains (T_m: Nakase and Komagata 1971e); 42.9–43.4, CBS 707 and 2 additional strains (BD: Price et al. 1978); 44.0–44.5, CBS 707 and 6 additional strains (BD: Kurtzman 1990c).

Origin of the strains studied: CBS 707 (NRRL Y-1559), sediment of a peppermint beverage; CBS 6544 (NRRL Y-17054), oak tree (*Quercus* sp.), California, U.S.A. (1); CBS 6711 (NRRL Y-17055), orange drink, Iraq (1); CBS 4506 (NRRL Y-7434), fruit fly (*Drosophila* sp.), California (1); CBS 7004 (NRRL Y-11844), type strain of *Saccharomyces astigiensis*, alpechín, Spain; CBS 7005 (NRRL Y-11847), type strain of *Saccharomyces albasitensis*, alpechín, Spain; CBS 4686 (NRRL Y-12620), type strain of *Saccharomyces nilssoni* var. *malicitensis*, alpechín, Spain.

Type strain: CBS 707.

Comments: Kurtzman (1990c) determined the extent of nDNA relatedness between strains of *Z. fermentati* and found the species to be comprised of two populations each having high intragroup complementarity (95%) but diminished intergroup relatedness (70–77%). The first group included the type strain as well as CBS 6544 and CBS 6711. The second group was comprised of the type strains of *Saccharomyces montanus* (CBS 4506), *S. astigiensis* (CBS 7004), *S. albasitensis* (CBS 7005) and *S. nilssoni* var. *malicitensis* (CBS 4686). The divergent population has been maintained within *Z. fermentati*, but genetic crosses might show members of this group to be a sibling species of *Z. fermentati*.

57.5. *Zygosaccharomyces florentinus* Castelli ex Kudryavtsev (1960)

Synonyms:

Saccharomyces florentinus (Castelli ex Kudryavtsev) Lodder & Kreger-van Rij (1952)

Torulaspora florentina (Castelli ex Kudryavtsev) van der Walt & E. Johannsen (1975a)

Zygosaccharomyces eupagycus Sacchetti ex Kudryavtsev (1960)

Saccharomyces eupagycus (Sacchetti ex Kudryavtsev) van der Walt (1970d)

Torulaspora eupagyca (Sacchetti ex Kudryavtsev) van der Walt & E. Johannsen (1975a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.1–7.0) × (2.5–7.1) μm, and occur singly, in pairs, or occasionally in small clusters. Growth is butyrous and white to tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional outgrowths of relatively simple, moderately branched pseudohyphae. True hyphae are not formed. Aerobic growth is butyrous, tannish-white, glistening, and with a smooth to finely striated surface. Margins are finely lobed. A faint, ester-like odor is present.

Formation of ascospores: Asci are persistent and generally form following conjugation between individual cells, but occasional asci may be unconjugated or show conjugation between a cell and its bud. One to four smooth, spheroidal to subspheroidal ascospores form in each ascus. The species appears to be homothallic.

Ascospores were observed on YM, corn meal, and 5% malt extract agars after 1–4 weeks at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	+	D-Gluconate	w/–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Gelatin liquefaction	–
5-Keto-D-gluconate	–	GTYE/1% acetic acid[1]	–
Saccharate	–	0.1% Cycloheximide	+
10% NaCl/5% glucose	–	w/o Inositol	+
Starch formation	–	Growth at 37°C	–

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: 6, AJ 4100 (Yamada et al. 1976b).

Mol% G+C: 42.4, CBS 746 and CBS 748 (BD: Price et al. 1978); 43.1, 42.8, CBS 746 and CBS 6081 (BD: Kurtzman 1990c).

Origin of the strains studied: CBS 746 (NRRL Y-1560), from grape must, Italy; CBS 748 (NRRL Y-1558), type strain of *Z. eupagycus,* orange drink, Italy; CBS 6081 (NRRL Y-12642), mountain laurel (*Rhododendron* sp.), Japan.

Type strain: CBS 746.

Comments: Price et al. (1978) showed 100% nDNA relatedness between the type strains of *Z. florentinus* and *Z. eupagycus,* thus demonstrating the conspecificity of the two taxa.

57.6. *Zygosaccharomyces mellis* Fabian & Quinet (1928)

Synonyms:

Saccharomyces mellis (Fabian & Quinet) Lodder & Kreger-van Rij (1952)
Saccharomyces bisporus (H. Naganishi) Lodder & Kreger-van Rij var. *mellis* (Fabian & Quinet) van der Walt (1970d)
Zygosaccharomyces mellis-acidi von Richter (1912)
Zygosaccharomyces nadsonii Guilliermond (1918)
Zygosaccharomyces nussbaumeri Lochhead & Heron (1929)
Zygosaccharomyces amoeboideus Kroemer & Krumbholz (Krumbholz 1931)
Zygosaccharomyces polymorphus Kroemer & Krumbholz f. *stellata* Krumbholz (1931)
Saccharomyces rouxii Boutroux var. *polymorphus* (Kroemer & Krumbholz) Lodder & Kreger-van Rij (1952)
Zygosaccharomyces perspicillatus Sacchetti (1932c)
Zygosaccharomyces ravennatis Sacchetti (1932c)
Kluyveromyces osmophilus Kreger-van Rij (1966a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, or infrequently cylindroidal, (2.5–2.9)×(3.0–6.2) μm, and occur singly, in pairs, and in small clusters. Growth is butyrous and white to tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows occasional branched chains of elongated cells, but neither well-developed pseudohyphae nor true hyphae occur. Aerobic growth is butyrous, white, with surfaces that are smooth or irregular, and either dull or glistening. Margins are finely or irregularly lobed. A faint, acidic, ester-like odor is present.

Formation of ascospores: Ascospores were not observed in the present study.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	w/–	Trehalose	–
Maltose	+/w		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	w/–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	GTYE/1% acetic acid[1]	–
10% NaCl/5% glucose	+	0.1% Cycloheximide	–
12.5% NaCl/5% glucose	+/w	w/o Inositol	+
15% NaCl/5% glucose	w/–	Growth at 37°C	–
16% NaCl/5% glucose	–		

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: 6, AJ 5262, AJ 5263 (Yamada et al. 1976b).

Mol% G+C: 41.2–41.8, CBS 736 and 6 additional strains (BD: Kurtzman 1990c).

Origin of the strains studied: CBS 736 (NRRL Y-12628), from honey; NRRL Y-56, NRRL Y-58, unknown sources, Tanner Collection, U.S.A.; NRRL Y-1024, honey?, Lochhead strain D-1; NRRL Y-1601 (CBS 684), honey, von Richter, Germany?; NRRL Y-17113, NRRL Y-17114, alpechín, Spain.

Type strain: CBS 736.

Comments: *Z. mellis* previously has been considered a variety of *Z. bisporus* (van der Walt 1970d) and as a synonym of *Z. rouxii* (Yarrow 1984d). Because of the difficulty in identifying strains of *Zygosaccharomyces* using phenotypic tests, Kurtzman (1990c) examined relationships among members of the genus from extent of nDNA complementarity. The study revealed that *Z. mellis* is a distinct species. Additionally, two of the strains (NRRL Y-56 and NRRL Y-1601) showed ca. 75% relatedness with the type strain but 97% relatedness with each other. These two isolates are presently regarded as divergent strains of *Z. mellis,* rather than as members of a sibling species.

Separation of *Z. mellis* and *Z. rouxii* is difficult using standard growth tests. *Z. rouxii* is slightly more tolerant of sodium chloride than is *Z. mellis,* thus allowing their separation on a medium containing 16% NaCl with 5% glucose (Kurtzman 1990c). Because the difference in sodium chloride tolerance between the two species is rather slight, reference strains need to be included for comparison.

57.7. *Zygosaccharomyces microellipsoides* (Osterwalder) Yarrow (von Arx et al. 1977)

Synonyms:

Saccharomyces microellipsoides (as *S. microellipsodes*) Osterwalder (1924a)

Torulaspora microellipsoides (Osterwalder) van der Walt & E. Johannsen (1975a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.8–6.9)×(2.1–7.1) µm, and occur singly, in pairs, and in small clusters. Growth is butyrous and white to tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass shows infrequent branched chains of elongated cells, but neither well-developed pseudohyphae nor true hyphae occur. Aerobic growth is butyrous, tannish-white, dull glistening, and with a finely lobed margin. A faint, acidic, ester-like odor is present.

Formation of ascospores: Asci are persistent and generally form following conjugation between independent cells. The asci form one to four smooth, spheroidal ascospores. The species is probably homothallic.

Ascospores were observed on YM agar after 2–4 weeks at 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+/w
Sucrose	+	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	+	D-Gluconate	s
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Gelatin liquefaction	–
5-Keto-D-gluconate	–	GTYE/1% acetic acid[1]	–
Saccharate	–	0.1% Cycloheximide	–
10% NaCl/5% glucose	–	w/o Inositol	+
Starch formation	–	Growth at 37°C	–

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: 6, AJ 4091, AJ 5292 (Yamada et al. 1976b).

Mol% G+C: 39.5, 39.3, AJ 4091, AJ 5292 (T_m: Nakase and Komagata 1971e); 40.3, CBS 427 (BD: Price et al. 1978); 41.2, 41.5, CBS 427, CBS 6142 (BD: Kurtzman 1990c).

Origin of the strains studied: CBS 427 (NRRL Y-1549), apple juice, Germany; CBS 2734 (NRRL Y-17057), berries of black currants (*Ribes nigrum*), Denmark; CBS 6142 (NRRL Y-17058), CBS 6143 (NRRL Y-17059), tea-beer, Finland; CBS 6641 (NRRL Y-17060), exudate of sandalwood (*Myoporum* sp.), Hawaii, U.S.A.; CBS 6762 (NRRL Y-17061), lemonade, Switzerland.

Type strain: CBS 427.

57.8. *Zygosaccharomyces mrakii* Capriotti (1958c)

Synonyms:

Saccharomyces mrakii (Capriotti) van der Walt (1970c)

Torulaspora mrakii (Capriotti) van der Walt & E. Johannsen (1975a)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.1–7.0)×(3.0–7.5) µm, and single or in pairs. Growth is butyrous and white to tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass may show poorly differentiated pseudohyphae, but true hyphae are absent. Aerobic growth is butyrous, glistening, and tannish-white with some reddish-brown, highly ascosporogenous areas. Margins are entire to irregularly lobed. A faint, acidic odor is present.

Formation of ascospores: Asci are persistent and may be unconjugated or arise following conjugation

between independent cells. Each ascus forms one to four smooth, spheroidal ascospores. The species is probably homothallic.

Ascospores were observed on YM agar after 1–4 weeks at 25°C and on Difco yeast morphology agar after 1 week at 25°C. The latter medium gave highly ascosporogenous cultures.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Gelatin liquefaction	–
5-Keto-D-gluconate	–	GTYE/1% acetic acid[1]	–
Saccharate	–	0.1% Cycloheximide	+
10% NaCl/5% glucose	v	w/o Inositol	+
12.5% NaCl/5% glucose	–	Growth at 37°C	–
Starch formation	–		

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: Not determined.

Mol% G + C: 41.2, 41.3, CBS 4218 and 1 additional strain (BD: Price et al. 1978); 42.2, 42.1, CBS 4218, CBS 4219 (BD: Kurtzman 1990c).

Origin of the strains studied: CBS 4218 (NRRL Y-12654), CBS 4219 (NRRL Y-12655), both from silage, Italy.

Type strain: CBS 4218.

57.9. *Zygosaccharomyces rouxii* (Boutroux) Yarrow (von Arx et al. 1977)

Synonyms:

Saccharomyces rouxii Boutroux (1884)
Zygosaccharomyces barkeri Saccardo & Sydow (1902)
?*Saccharomyces soya* Saito (1907)
?*Zygosaccharomyces soya* (Saito) Takahashi & Yukawa (1915)
?*Zygosaccharomyces japonicus* Saito var. *soya* (Saito) Dekker (Stelling-Dekker 1931)
Zygosaccharomyces japonicus Saito (1909)
Zygopichia japonica (Saito) Klöcker (1924)
?*Zygosaccharomyces major* Takahashi & Yukawa (1915)
?*Zygosaccharomyces salsus* Takahashi & Yukawa (1915)
?*Zygopichia salsa* (Takahashi & Yukawa) Klöcker (1924)
?*Zygosaccharomyces dairensis* H. Naganishi (1928)
Zygosaccharomyces vini H. Naganishi (1928)
Zygosaccharomyces cavarae Rodio var. *beauveriei* Beauverie (1929)
Zygosaccharomyces richteri Lochhead & Heron (1929)
?*Zygosaccharomyces cavarae* Rodio var. *amoeboideus* Lodder (1932)
Zygosaccharomyces gracilis Karamboloff & Krumbholz (1931)
Zygosaccharomyces polymorphus Kroemer & Krumbholz (Krumbholz 1931)
Zygosaccharomyces polymorphus Kroemer & Krumbholz f. *craterica* Krumbholz (1931)
Zygosaccharomyces polymorphus Kroemer & Krumbholz f. *typica* Krumbholz (1931)
?*Saccharomyces rouxii* Boutroux var. *polymorphus* (Kroemer & Krumbholz) Lodder & Kreger-van Rij (1952)
Zygosaccharomyces variabilis Kroemer & Krumbholz (Krumbholz 1931)
Zygosaccharomyces rugosus Lochhead & Farrell (Lochhead 1942)
?*Zygosaccharomyces nectarophilus* Lochhead & Farrell (Lochhead 1942)
Zygosaccharomyces gracilis Karamboloff & Krumbholz ssp. *italicus* Sacchetti (1932d)
Zygosaccharomyces citrus Lodder (1932)
Zygosaccharomyces major Takahashi & Yukawa var. *threntensis* Lodder (1932)
Zygosaccharomyces felsineus Sacchetti (1932d)
?*Zygosaccharomyces miso* β Mogi (1938b)
?*Saccharomyces miso* δ Mogi (1939)
?*Zygosaccharomyces nukamiso* Otani (1939) nom. nud.
?*Zygosaccharomyces halomembranis* Etchells & T.A. Bell (1950a)
?*Saccharomyces rouxii* Boutroux var. *halomembranis* (Etchells & T.A. Bell) Onishi (1957)
?*Saccharomyces acidifaciens* (Nickerson) Lodder & Kreger-van Rij var. *halomembranis* (Etchells & T.A. Bell) Onishi (1957)
Torulopsis osloensis Dietrichson (1954) nom. nud.
Saccharomyces osmophilus Barre & Galzy (1960) nom. nud.
?*Torulopsis mogii* Vidal-Leiria (1966a)
Saccharomyces bailii Lindner var. *osmophilus* van der Walt (1970d)
?*Candida placentae* S. Goto (1979b)
Saccharomyces placentae S. Goto (1980)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to cylindroidal, (3.0–7.8)×(3.5–8.1) μm, and occur singly, in pairs, or occasionally in small clusters. Growth is butyrous and white to tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass may show branched, but poorly differentiated pseudohyphae. True hyphae are not formed. Aerobic growth is butyrous, white to tannish-white, with surfaces smooth and glistening or finely convoluted and dull. Margins are entire or finely lobed. A faint, acidic odor is present.

Formation of ascospores: Asci are persistent and form one to four spheroidal to ovoidal ascospores that may appear smooth or slightly roughened. Ascosporulation may be preceded by conjugation between two cells or between a cell and its bud. (Fig. 198). Additionally, Wickerham and Burton (1960) reported some strains to represent heterothallic mating types.

Ascospores were observed on YM, Gorodkowa and 5% malt extract agars after 3–15 days at either 15° or 25°C.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	v	Trehalose	v
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	w/–	Glycerol	+/w
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	+/w	Galactitol	–
Lactose	–	D-Mannitol	+/w
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	w/–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	18% NaCl/5% glucose	v
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Gelatin liquefaction	–
10% NaCl/5% glucose	+	GTYE/1% acetic acid[1]	–
12.5% NaCl/5% glucose	+	0.1% Cycloheximide	–
15% NaCl/5% glucose	+/w	w/o Inositol	+
16% NaCl/5% glucose	+/w	Growth at 37°C	v
17% NaCl/5% glucose	v	Growth at 40°C	–

[1] Growth on GTYE agar with 1% acetic acid (see Table 46).

Co-Q: 6, CBS 732 and 2 additional strains (Yamada et al. 1976b).

Mol% G+C: 40.0, CBS 732 and 2 additional strains (T_m: Nakase and Komagata 1971e); 41.3–42.3, CBS 732 and 6 additional strains (BD: Kurtzman 1990c).

Origin of the strains studied: CBS 732 (NRRL Y-229), from grape must, Italy; CBS 742 (NRRL Y-998), from honey, Canada; NRRL Y-1053, unknown; CBS 4837 (NRRL Y-2547), CBS 4838 (NRRL Y-2548), miso, Japan; CBS 8000 (NRRL Y-12616), type strain of *Saccharomyces placentae,* sweet cream sake, Japan; CBS 4021 (NRRL Y-12622), type strain of *Saccharomyces bailii* var. *osmophilus*, soy mash, Japan; NRRL Y-12691, chocolate syrup, Pennsylvania, U.S.A.

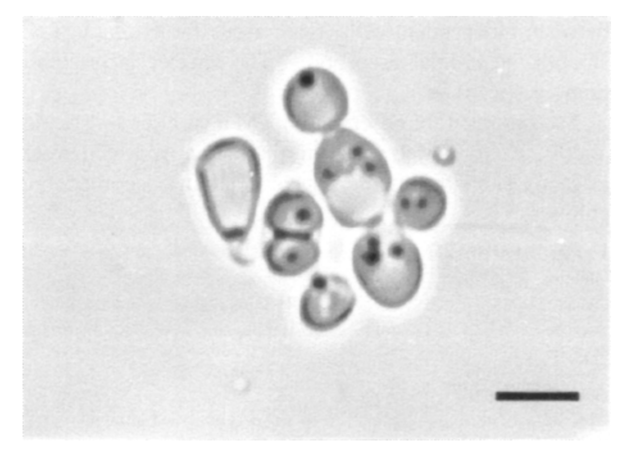


Fig. 198. *Z. rouxii,* CBS 732. Ascus with ascospores, after 2 weeks on 5% malt extract agar at 15°C. Bar = 5 µm.

Complementary mating types: CBS 4837 and CBS 4838.

Type strain: CBS 732, neotype designated by Lodder and Kreger-van Rij (1952). The type strain of *Zygosaccharomyces gracillus* ssp. *italicus.*

Comments: On the basis of phenotypic similarity, *Candida mogii* was believed to represent the anamorph of *Z. rouxii* (Yarrow 1984d). Comparisons of nDNA relatedness showed that the two taxa are separate species (Kurtzman 1990c).

Comments on the genus

The nine species presently accepted in *Zygosaccharomyces* are genetically distinct from one another on the basis of low nDNA relatedness (Kurtzman 1990c). James et al. (1994b, 1996) examined interspecific relationships among species of *Zygosaccharomyces* from comparisons of 18S rRNA gene sequences as well as from nucleotide diversity in the two internal transcribed spacer regions (ITS1, ITS2). Species of *Torulaspora* were included in the ITS study and shown to arise from within *Zygosaccharomyces.* As discussed under *Torulaspora,* circumscription of *Torulaspora* as well as *Saccharomyces* and *Zygosaccharomyces* is likely to change once additional molecular comparisons are done.

58. *Zygozyma* van der Walt & von Arx

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is by multilateral budding, but occasionally by rudimentary septation with the formation of plasmodesmal canals. Cells are hyaline, encapsulated and spherical to ovoidal. Pseudohyphae and septate hyphae are absent. Colonies are watery, becoming mucoid to glutinous.

Asci are saccate, one-to multispored, lytic, arising either from abstricted enlarging evaginations of single cells, from conjugating cells or direct transformation of cells. Ascospores are allantoid to cymbiform, smooth, and amber-colored, and agglutinate when released.

Sugars are not fermented. Nitrate is not assimilated. Extracellular amyloid material is formed. Diazonium blue B reaction is negative.

Type species

Zygozyma oligophaga van der Walt & von Arx

Species accepted

1. *Zygozyma arxii* van der Walt, M.Th. Smith & Y. Yamada (1989)
2. *Zygozyma oligophaga* van der Walt & von Arx (1987)
3. *Zygozyma smithiae* van der Walt, Wingfield & Y. Yamada (1990)
4. *Zygozyma suomiensis* M.Th. Smith, van der Walt & Y. Yamada (1989)

Key to species

See Table 47.

1.	a	Trehalose assimilated	*Z. arxii*:	p. 433
	b	Trehalose not assimilated → 2		
2(1).	a	Sucrose assimilated	*Z. smithiae*:	p. 434
	b	Sucrose not assimilated → 3		
3(2).	a	L-Arabinose assimilated	*Z. suomiensis*:	p. 435
	b	L-Arabinose not assimilated	*Z. oligophaga*:	p. 434

Table 47
Key characters of species assigned to the genus *Zygozyma*

Species	Assimilation		
	Sucrose	Trehalose	L-Arabinose
Zygozyma arxii	–	+	+
Z. oligophaga	–	–	–
Z. smithiae	+	–	–
Z. suomiensis	–	–	+

Systematic discussion of the species

58.1. *Zygozyma arxii* van der Walt, M.Th. Smith & Y. Yamada (van der Walt et al. 1989d)

Growth in malt extract: After 3 days at 25°C, the cells are globose to ellipsoidal, (3.0–7.0)×(2.5–5.5) μm, encapsulated, reproduce by multilateral budding, and occur singly or in pairs. Growth is scant and a slight sediment is formed. After 1 month at room temperature, a slight ring and sediment are present.

Growth on malt agar: After 3 days at 25°, the cells have the same appearance as in malt extract. The streak culture is partly hyaline and viscous, partly butyrous and creamish-gray, smooth, glistening, and with an entire margin. After 4 weeks at room temperature, the culture is butyrous, brownish-cream, smooth, somewhat shiny, slightly raised along the center, and with an entire margin.

Dalmau plate cultures on corn meal agar: After

10 days at 25°C, neither hyphae nor pseudohyphae are formed.

Formation of ascospores: Asci are unattached, conjugated, often dumbbell-shaped, (10.0–15.0, rarely up to 19.0)×(2.0–6.0) µm, one- to multispored, evanescent, and generally arise from the fusion of protuberances formed by two free cells. Automictic asci are less common. Unconjugated, free asci are rarely observed. Ascospores are allantoid to cymbiform, (2.5–4.5)×(1.0–1.5) µm, glabrous amber-colored, and conglutinate when liberated.

Ascus formation was observed on 2% malt agar, V8 agar and 1/10 diluted V8 agar. Sporulation is slow, requiring 2–5 weeks at 15–18°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	+	Growth at 30°C	+
0.1% Cycloheximide	+	Growth at 35°C	–

Co-Q: 9 (van der Walt et al. 1989d).
Mol% G+C: 47.4, CBS 7333 (T_m: van der Walt et al. 1989d).

Origin of the strains studied: CBS 7333, forest soil, South Africa, J.P. van der Walt.

Type strain: CBS 7333 (ATCC 76360), isolated by J.P. van der Walt.

58.2. *Zygozyma oligophaga* van der Walt & von Arx (van der Walt et al. 1987b)

Growth in malt extract: After 3 days at 25°C, the cells are globose to ellipsoidal, (3.0–7.0)×(2.5–5.5) µm, encapsulated, reproduce by multilateral budding, and occur singly or in pairs. Growth is scant and a slight sediment is formed. After 1 month at room temperature, a slight ring and sediment are present.

Growth on malt agar: After 3 days at 25°C, the cells have the same appearance as in malt extract. The streak culture is partly hyaline to slightly creamish-gray and viscous. After 4 weeks at room temperature, the culture is butyrous, brownish-cream, smooth, somewhat shiny, slightly raised along the center, with an entire margin.

Dalmau plate cultures on corn meal agar: After 10 days at 25°C, neither pseudohyphae nor septate hyphae are formed. Asci may be present.

Formation of ascospores: Asci are saccate, (9.0–15.0, rarely up to 25.0)×(4.0–7.0, rarely up to 11.0) µm, four- to multispored, evanescent and arise from inflated protuberances produced either by adjacent or non-adjacent aggregated cells. Ascospores are allantoid or cymbiform, (2.5–4.0×1.0–1.5) µm, smooth, amber-colored and conglutinate when liberated.

Sporulation is abundant on 2% malt agar and V8 agar after 3–4 days at 25°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	+
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	v
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	+	Growth at 30°C	+
0.1% Cycloheximide	+	Growth at 35°C	–

Co-Q: 8 (van der Walt et al. 1987b).
Mol% G+C: 41.7, CBS 7107 (T_m: van der Walt et al. 1987b).

Origin of the strains studied: CBS 7107 (ATCC 76359), frass of a bark beetle infesting moribund *Ficus tripoda,* South Africa, J.P. van der Walt; CBS 7406, frass of *Crossotarsus externedentatus* in *Macaranga capensis,* South Africa, J.P. van der Walt.

Type strain: CBS 7107 (ATCC 76359), isolated by J.P. van der Walt.

58.3. *Zygozyma smithiae* van der Walt, Wingfield & Y. Yamada (1990b)

Growth in malt extract: After 3 days at 25°C, the cells are globose to ellipsoidal, (3.0–7.0)×(2.5–5.5) µm, encapsulated, reproduce by multilateral budding, occasionally on a broad base, and occur singly or in pairs. Growth is scant and a slight sediment is formed. After 1 month at room temperature, a slight ring and sediment are present.

Growth on malt agar: After 3 days at 25°C, the cells have the same appearance as in malt extract. The streak culture is watery to mucoid, hyaline to creamish-opaque, smooth, and shiny with an entire margin. After 4 weeks at room temperature, the culture is butyrous, hyaline to brownish-cream, smooth, somewhat shiny, slightly raised along the center, and with an entire margin.

Dalmau plate cultures on corn meal agar: After 10 days at 25°C, neither pseudohyphae nor septate hyphae are formed.

Formation of ascospores: Asci are unconjugated, attached, globose allantoid or amoeboid, multispored, (9.0–18.0×3.0–9.0) µm, evanescent, and arise by the direct transformation of presumed diploid, aggregated, vegetative cells. Ascospores are allantoid to cymbiform, (2.5–3.5×1.0–1.5) µm, glabrous, amber-colored, and conglutinate when liberated.

Ascus formation was observed on 2% malt agar after 6 days at room temperature.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	+	Growth at 30°C	+
0.1% Cycloheximide	+	Growth at 35°C	–

Co-Q: 9 (van der Walt et al. 1990b).

Mol% G+C: 55.7, CBS 7407; 55.3, CBS 7408 (T_m: van der Walt et al. 1990b).

Origin of the strains studied: CBS 7407, CBS 7408, frass of *Crossotarus externedentatus* (ambrosia beetle) in *Macaranga capensis,* South Africa, J.P. van der Walt.

Type strain: CBS 7407 (ATCC 76360), isolated by J.P. van der Walt.

58.4. *Zygozyma suomiensis* M.Th. Smith, van der Walt & Y. Yamada (Smith et al. 1989b)

Growth in malt extract: After 3 days at 25°C, the cells are globose to ellipsoidal, (3.0–7.0)×(2.5–5.5) µm, encapsulated, reproduce by multilateral budding, and occur singly or in pairs. Growth is scant and a slight sediment is formed. After 1 month at room temperature, a slight ring and sediment are present.

Growth on malt agar: After 3 days at 25°C, the cells have the same appearance as in malt extract. The streak culture is viscous to mucoid, partly hyaline, partly creamish-gray, smooth, glistening, and with an entire margin. After 4 weeks at room temperature, the culture is butyrous, brownish-cream, smooth, somewhat shiny, slightly raised along the center, and with an entire margin.

Dalmau plate cultures on corn meal agar: After 10 days at 25°C, neither pseudohyphae nor septate hyphae are produced.

Formation of ascospores: Asci arise from abstricted inflated evaginations on single cells or from individual cells in aggregations. Asci are saccate, (5.0–11.0×4.5–7.0) µm, persistent and contain four to numerous (up to 12) ascospores. Ascospores are allantoid to oblong with obtuse ends, (2.0–4.5×1.0–2.0) µm, and glabrous.

Asci are produced after 6 weeks on Difco malt (5%)–agar (3%) at 15°C.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	s	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	s	D-Mannitol	s
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	s
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	s
D-Arabinose	+	Inositol	–
D-Ribose	s	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	+	Growth at 30°C	+
0.1% Cycloheximide	+	Growth at 35°C	–

Co-Q: 8 (Smith et al. 1989b).

Mol% G+C: 45.1, CBS 7251 (T_m: Smith et al. 1989b).

Origin of the strains studied: CBS 7251, skin lesion of a cow, Finland, E. Tunnela.

Type strain: CBS 7251 (ATCC 66456), isolated by E. Tunnela.

Comments on the genus

In 1987, van der Walt et al. (1987b) introduced the genus *Zygozyma* to accommodate yeast strains associated with arboricole *Coleoptera* in Northern Natal. These

strains formed mucoid colonies on solid medium similar to species of *Lipomyces* and *Myxozyma* and produced asci with allantoid ascospores resembling those of the hyphal genus *Dipodascopsis.* Differences in ascospore shape and absence of septate hyphae were considered decisive characteristics to assign these isolates to a new genus, with the species *Z. oligophaga.* Van der Walt et al. (1991) emended the description of *Zygozyma* following demonstration by transmission electron microscopy of the occurrence of plasmodesmal canals in cells of *Z. oligophaga* and *Z. smithiae,* species that show budding on a broad base.

Part Vc

Descriptions of anamorphic ascomycetous genera and species

Contents

59. *Aciculoconidium* D.S. King & S.-C. Jong

M.Th. Smith

Diagnosis of the genus

Budding cells are ovoidal or ellipsoidal and single, in short chains or clusters. Mycelium is present and consists of branched septate hyphae with blastoconidia. Terminal needle-shaped conidia that are rounded at the base and pointed at the top are also present.

Ascospores and asexual endospores are not produced.

Sugars are fermented. Nitrate is not assimilated. Diazonium blue B reaction is negative.

Type species

Aciculoconidium aculeatum (Phaff, M.W. Miller & Shifrine) D.S. King & S.-C. Jong

Species accepted

1. *Aciculoconidium aculeatum* (Phaff, M.W. Miller & Shifrine) D.S. King & S.-C. Jong (1976)

Systematic discussion of the species

59.1. *Aciculoconidium aculeatum* (Phaff, M.W. Miller & Shifrine) D.S. King & S.-C. Jong (1976a)

Synonym:
Trichosporon aculeatum Phaff, M.W. Miller & Shifrine (1956)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, budding cells and mycelium are formed. Budding cells are ovoidal or ellipsoidal, (3.5–8.5)×(4.0–15.0) μm, and single, in chains or in small clusters. Mycelium consists of thin as well as broad hyphae that are septate. Needle-shaped cells, which are rounded at the base and pointed at the other end, frequently arise from blastospores from which they easily separate (Fig. 199). An irregular ring and a mycelial sediment are present. After one month at 25°C, mycelial growth occurs throughout.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is cream-colored, smooth to slightly wrinkled, glossy to dull, and tough.

Dalmau plate cultures on corn meal agar: Abundant true mycelium with blastospores is formed. Blastospores are borne singly or successively in chains, and apically-produce needle-shaped cells, (0.5–2.0)×(6.0–21.5) μm, which are rounded at the base and pointed at the other end (Fig. 200), are also present. The mycelium may disarticulate.

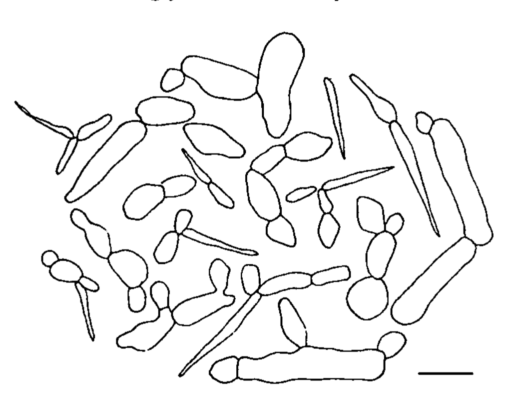


Fig. 199. *A. aculeatum*, CBS 5578. Budding cells in glucose–yeast extract–peptone water after 3 days at 25°C. Bar = 10 μm.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	v	Trehalose	w/s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	s	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	+/w	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

50% Glucose	–	Growth at 37°C	–
0.1% Cycloheximide	+		

Co-Q: 9 (Yamada et al. 1982).

Mol% G+C: 55.0, CBS 2282 (NRRL YB-4297) (T_m: Dupont and Hedrick 1971).

Origin of the strains studied: CBS 5578, *Drosophila occidentalis*, do Carmo-Sousa; CBS 2282, *D. occidentalis*, Phaff; CBS 5293, *D. pinicola*, California, U.S.A., do Carmo-Sousa.

Type strain: CBS 5578 (ATCC 28680), isolated by Phaff.

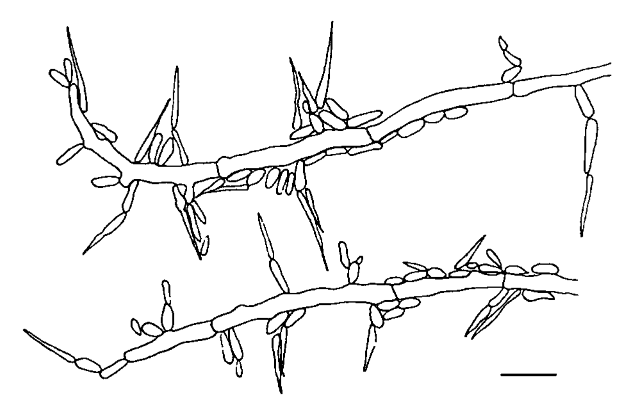


Fig. 200. *A. aculeatum,* CBS 5578. Hyphae in Dalmau plate culture on corn meal agar after 7 days at 25°C. Bar = 10 μm.

Comments on the genus

King and Jong (1976b) examined the process of arthroconidia formation in *Trichosporon.* On the basis of differences in this process they removed *T. aculeatum* from the genus. Since these authors considered the needle-shaped blastoconidia an important characteristic by which *T. aculeatum* differed from previously described species, the new genus *Aciculoconidium* was introduced to accommodate *T. aculeatum* (King and Jong 1976a).

Some comments on the type strain designated by King and Jong (1976a) have to be made. These authors stated in their publication that two subcultures of the type strain isolated from *Drosophila pinicola* were examined. However, the culture chosen by Phaff et al. (1956) as the type and labelled in the original publication as F-0145A had been isolated from *Drosophila occidentalis* (Phaff, personal communication). To this culture the number CBS 5578 was assigned. The culture ATCC 1550 = NRRL YB-4297 considered by King and Jong as an iso-type strain is a subculture of Phaff's isolate from *Drosophila pinicola* (Kurtzman, personal communication). Since subcultures of the authentic strain still exist, the strain CBS 5578 = ATCC 28680 is the type strain.

60. *Arxula* van der Walt, M.Th. Smith & Y. Yamada

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is by multilateral budding. Pseudohyphae, septate hyphae and arthroconidia are abundantly formed. Hyphae are narrow, not spreading, disarticulating into squat arthroconidia. Blastoconidia may be formed on very short denticles of hyphae. Colonies are restricted, dry, dull, white to cream-colored.

Ascospores are not produced.

Fermentation may be present. Nitrate is assimilated. Cultures are xerotolerant. Extracellular amyloid compounds are not produced. Septa have micropores. Diazonium blue B reaction is negative.

Type species

Arxula terrestris (van der Walt & E. Johannsen) van der Walt, M.Th. Smith & Y. Yamada

Species accepted

1. *Arxula adeninivorans* (Middelhoven, Hoogkamer-Te Niet & Kreger-van Rij) van der Walt, M.Th. Smith & Y. Yamada (1990)
2. *Arxula terrestris* (van der Walt & E. Johannsen) van der Walt, M.Th. Smith & Y. Yamada (1990)

Key to species

1. a Melibiose assimilated .... *A. adeninivorans*: p. 441
   b Melibiose not assimilated .... *A. terrestris*: p. 442

Systematic discussion of the species

60.1. *Arxula adeninivorans* (Middelhoven, Hoogkamer-Te Niet & Kreger-van Rij) van der Walt, M.Th. Smith & Y. Yamada (1990c)

Synonym:

Trichosporon adeninovorans Middelhoven, Hoogkamer-Te Niet & Kreger-van Rij (1984)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, budding cells are short-ovoidal to long-ovoidal, (2.2–3.6)×(2.4–5.6) µm. Branched, septate hyphae, 2.4 µm in diameter, and arthroconidia are also present. A sediment and a white, dry pellicle are formed.

Growth on glucose–yeast extract–peptone agar: After one month the streak culture is yellowish-white, partly soft, partly tough and restricted. The edge is fringed with mycelium.

Dalmau plate cultures on potato- and corn meal agar: True, branched, septate mycelium is abundantly formed. It may disarticulate into arthroconidia. Spheroidal or short-ovoidal blastoconidia occur in chains, sometimes on short denticles on the hyphae, but generally between, not at the septa. Aerial hyphae occur.

Formation of endospores: Asexual, spheroidal to ovoidal, endospores of variable size and number (3–6), are observed on Difco malt agar.

Fermentation:

Glucose	s	Lactose	–
Galactose	s	Raffinose	s
Sucrose	+	Trehalose	s
Maltose	s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	s	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	s
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	s	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

60% (w/w) glucose–yeast extract agar	+	Growth at 40°C	+
0.1% Cycloheximide	+		

Co-Q: 9 (van der Walt et al. 1990c).

Mol% G + C: Not determined.

Origin of the strains studied: CBS 7350, ensiled whole-crop chopped maize, Netherlands, W.J. Middelhoven; CBS 7370, soil, CBS 7377, garden soil, South Africa, J.P. van der Walt; CBS 7766, liver and intestines of *Heloderma suspectum*, Sweden, R. Mattson; CBS 8244, type strain of *Trichosporon adeninovorans*, soil, Netherlands, W.J. Middelhoven.

Type strain: CBS 8244, isolated by W.J. Middelhoven.

60.2. *Arxula terrestris* (van der Walt & E. Johannsen) van der Walt, M.Th. Smith & Y. Yamada (1990c)

Synonyms:
Trichosporon terrestre van der Walt & E. Johannsen (1975d)
Geotrichum terrestre (van der Walt & E. Johannsen) Weijman (1979b)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, branched, septate mycelium, (2.0–3.5) μm in diameter, arthroconidia and pseudomycelium are abundantly formed. Budding yeast cells are ovoidal, ellipsoidal to cylindrical, (2.5–6.5)×(3.0–11.0) μm, and occur singly, in pairs or small clusters (Fig. 201). A flocculent sediment and incomplete ring are present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°, the streak culture is cream-colored, dry, soft, restricted, raised and delicately wrinkled, with a lobate margin.

Dalmau plate cultures on potato- and corn meal agar: Pseudomycelium, septate hyphae and arthroconidia are abundantly formed. Blastoconidia occur on the hyphae at the septa or in between, and may be single or in short chains. Arthroconidia vary in length, and may be short and spheroidal.

Formation of endospores: Asexual endospores, which are formed by endoplasmic cleavage, were observed in old cultures on YM agar.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	s	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	s
Melezitose	–	Salicin	s
Inulin	–	D-Gluconate	+
Soluble starch	s	DL-Lactate	s
D-Xylose	+	Succinate	s
L-Arabinose	+	Citrate	s
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

60% (w/w) glucose–yeast extract agar	+	Growth at 37°C	+
		Growth at 40°C	–
0.1% Cycloheximide	+		

Co-Q: 9 (van der Walt et al. 1990c).
Mol% G + C: Not determined.

Origin of the strains studied: CBS 278.86 (CBS 6697), grassland soil; CBS 7376, soil, South Africa, J.P. van der Walt.

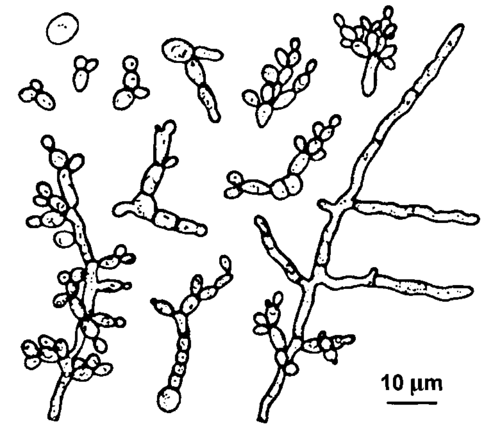


Fig. 201. *A. terrestris*, CBS 6697. Budding cells, pseudohyphae and septate hyphae. (After van der Walt and Johannsen 1975d.)

Type strain: CBS 278.86 (CBS 6697), isolated by J.P. van der Walt.

Comments on the genus

The genus *Arxula* was introduced by van der Walt et al. (1990c) to accommodate two arthroconidial, xerotolerant ascomycetous *Trichosporon* species, *T. terrestre* and *T. adeninovorans* of which the taxonomic position was unsettled. Weijman (1979b) revised the anamorphic arthroconidial genera *Trichosporon* and *Geotrichum* on the basis of whole-cell monosaccharide composition and restricted *Trichosporon* to basidiomycetous, arthroconidial anamorphs, and *Geotrichum* to ascomycetous, arthroconidial anamorphs. Consequently, *T. terrestre* was reclassified as *Geotrichum terrestre*. In 1984, Middelhoven et al. described the ascomycetous species *T. adeninovorans* with its uncommon ability to utilize certain purines (uric acid and adenine) as well as *n*-alkylamines (*n*-butylamine and pentylamine) as sole sources of carbon and nitrogen, a property which is shared with *G. terrestre*. De Hoog et al. (1986), in their revision of *Geotrichum*, excluded *G. terrestre* from this genus on the basis of its scant production of septate hyphae and its extremely yeastlike appearance. On the basis of its nutritional profile, *T. adeninovorans* was not transferred to *Geotrichum* by these authors. Since both species were excluded from *Geotrichum*, van der Walt et al. (1990c) followed the proposal of von Arx et al. (1977), and assigned *T. terrestre* and the related species to a new genus.

Studies on molecular relationships among hyphal ascomycetous yeasts and yeastlike taxa were published by Yamada and Nogawa (1990a) and Kurtzman and Robnett (1995). From the data of both studies it can be concluded that the genus *Arxula* is closely related to the teleomorph genus *Stephanoascus* and might represent anamorphs of this genus.

61. *Blastobotrys* von Klopotek

G.S. de Hoog and M.Th. Smith

Diagnosis of the genus

Colonies are restricted, dry, snow-white, and consist of compacted hyphae; budding cells are absent or present. Conidiophores are suberect, sympodial, and produce conidia in clusters on a rachis or alongside hyphae. Conidia are borne on pedicels or denticles, or are sessile. Primary conidia produce smaller, secondary conidia, either synchronously or sympodially in short, acropetal chains; primary conidia often bear setae. Septa have micropores. Teleomorph genus: *Stephanoascus.*

Sugars are often fermented. Nitrate is not assimilated. Diazonium blue B reaction is negative.

Type species

Blastobotrys nivea von Klopotek

Species accepted

1. *Blastobotrys arbuscula* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)
2. *Blastobotrys aristata* Marvanová (1976)
3. *Blastobotrys capitulata* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)
4. *Blastobotrys elegans* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)
5. *Blastobotrys gigas* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985) (anamorph of *Stephanoascus farinosus* de Hoog, Rantio-Lehtimäki & M.Th. Smith)
6. *Blastobotrys nivea* von Klopotek (1967)
7. *Blastobotrys proliferans* Marvanová (1976)

Key to species

Morphological key to species: See Table 48.

1. a Setae present on primary conidia → 2
 b Setae absent from primary conidia → 4
2(1). a Setae bearing new conidia similar to primary conidia, each with globose secondary conidia .... *B. proliferans*: p. 447
 b Setae sterile → 3
3(2). a Few primary conidia united on pedicels .... *B. aristata*: p. 444
 b Primary conidia clustered, sessile .... *B. capitulata*: p. 445
4(1). a Primary conidia formed on long, geniculate rachis .... *B. nivea*: p. 446
 b Primary conidia formed terminally in groups on poorly differentiated cells → 5
5(4). a Initial growth yeastlike → 6
 b Budding cells absent .... *B. elegans*: p. 446
6(5). a Primary conidia nearly globose, 4–6 μm diameter; secondary conidia globose; conidial apparatus stiff, poorly branched .... *B. arbuscula*: p. 444
 b Combination of preceding characters not found → 7
7(6). a Primary conidia barely different from secondary conidia, 2–3 μm wide .... anamorph of *Stephanoascus ciferrii*: p. 400
 b Primary conidia inflated, 3–6 μm wide .... *B. gigas*, anamorph of *Stephanoascus farinosus*: p. 446

Physiological key to species:

1. a Growth at 37°C → 2
 b Growth absent at 37°C → 5
2(1). a Fermentation of raffinose .... *B. proliferans*: p. 447
 b Raffinose not fermented → 3
3(2). a Fermentation of glucose → 4
 b Glucose not fermented .... *S. ciferrii*: p. 400
4(3). a Melibiose assimilated .... *B. nivea*: p. 446
 b Melibiose not assimilated .... *B. capitulata*: p. 445
5(1). a Fermentation of glucose → 6
 b Glucose not fermented .... *B. elegans*: p. 446

6(5). a Sucrose assimilated .... *B. aristata*: p. 444
b Sucrose not assimilated .... *B. arbuscula*, anamorph of *Stephanoascus farinosus*: p. 444

Table 48
Key characters of species in the genus *Blastobotrys*

Species	Fermentation		Assimilation							Growth at 37°C
	Glucose	Raffinose	Sucrose	Melibiose	D-Arabinose	L-Rhamnose	Ethanol	Salicin	Inositol	
Blastobotrys arbuscula	+	–	–	–	–	–	–	–	–	–
B. aristata	+	–	+	v	+	v	–	+	v	–
B. capitulata	+	–	v	–	v	+	+	+	+	+
B. elegans	–	–	–	–	–	–	+	v	–	–
B. nivea	+	–	v	+	+	–	+	+	+	+
B. proliferans	+	+	+	+	+	v	+	+	+	+

Systematic discussion of the species

61.1. *Blastobotrys arbuscula* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 1 mm in diameter, butyrous, and cream-colored. Budding cells are abundant and in coherent chains, composing star-shaped pseudomycelial microcolonies. Hyphae are hyaline, straight and stiff, 2.5–3.5 µm wide, and with clusters of minute conidium-bearing denticles at the apex and just below distal septa. Primary conidia are broadly ellipsoidal to subglobose, about 4–6 µm wide, without setae and produce densely crowded, globose secondary conidia, 2.5–3.5 µm diameter, in more or less synchronous order (Fig. 202).

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	+		

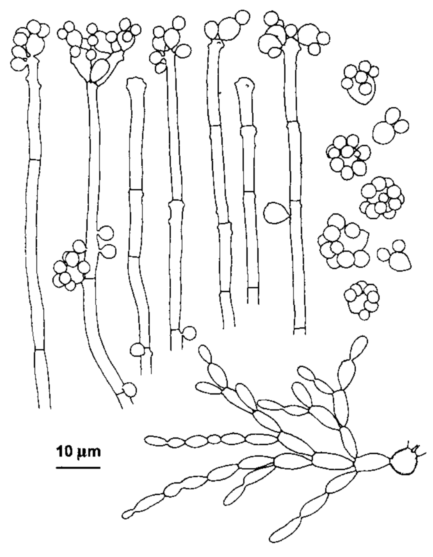


Fig. 202. *B. arbuscula*, CBS 227.83. Conidiophores with primary and secondary conidia and initial growth with multilateral budding. MEYA, 22°C, 10 days.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	–
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	–
Inulin	v	D-Gluconate	n
Soluble starch	+	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Growth at 37°C –
Co-Q: Not determined.
Mol% G + C: Not determined.

Origin of the strain studied: CBS 227.83, from house air, Tunnela, Finland.

Type strain: CBS 227.83.

Comments: The species is close to *B. gigas* de Hoog et al. (1985), which was recently reported to have a teleomorph similar to *Stephanoascus farinosus* de Hoog et al. (Traquair et al. 1988a). These taxa may belong to a single, psychrophilic, homothallic species, *S. farinosus*, which would be more variable in its morphological and physiological characters than was originally reported (de Hoog et al. 1985). Genomic studies are needed to clarify this matter.

61.2. *Blastobotrys aristata* Marvanová (1976)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 10 mm in diameter, dry, farinose, snow-white and consist of compact

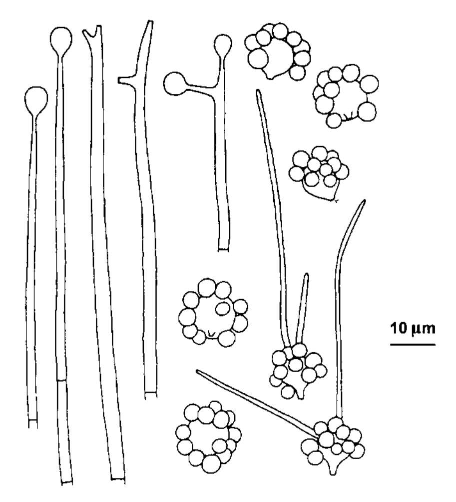


Fig. 203. *B. aristata*, CBS 521.75. Conidiophore tips with primary conidium initials and primary conidia with secondary conidia and setae. MEYA, 22°C, 10 days.

mycelium; budding cells are often absent. Hyphae are hyaline and 2–3 µm wide. Conidiophores are ascending, up to 100 µm long and bear 1–3 conidia at the apex; each is borne on a 2–4 µm long denticle. Primary conidia are pear-shaped, (3–8)×(4.5–9.0) µm, with 1–2 sterile setae and bear synchronously produced, densely crowded, globose secondary conidia of 1.5–3.5 µm diameter (Fig. 203).

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	+
Inulin	v	D-Gluconate	n
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	+	Inositol	v
D-Ribose	+	Hexadecane	n
L-Rhamnose	v	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Growth at 37°C –
Co-Q: Not determined.
Mol% G + C: Not determined.

Origin of the strain studied: CBS 521.75, from moldy plaster, Marvanová, Czechoslovakia.

Type strain: CBS 521.75.

Comments: Physiological characteristics are often difficult to judge because of formation of hyphal clumps. Primary conidia are borne on long, tapering pedicels which are formed singly or in small groups at the apex of conidiophores.

61.3. *Blastobotrys capitulata* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 5 mm in diameter, snow-white, dry, and evenly farinose. Budding cells are absent; hyphae are hyaline and 2.5–3.5 µm wide. Conidia are sessile and produced in dense clusters at the apex of poorly differentiated cells. Primary conidia are obovoidal, 5×10 µm, and bear 1–2 sterile, flexuose setae 0.5 µm wide and up to 200 µm long. Secondary conidia are synchronously produced in dense packets on primary conidia; they are hygrophobic, globose and 2.5–3.5 µm in diameter. Similar conidia are produced alongside the hyphae (Fig. 204).

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	v	D-Gluconate	n
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Growth at 37°C +
Co-Q: Not determined.
Mol% G + C: Not determined.

Origin of the strain studied: CBS 287.82, from rotting *Euphorbia ingens*, van der Walt, South Africa; from straw.

Type strain: CBS 287.82.

Comments: The closely aggregated, sessile primary conidia that bear very thin, flexuose, sterile setae are characteristic of this species.

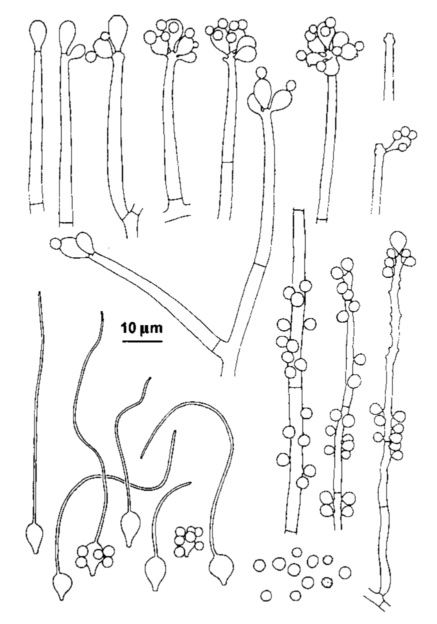


Fig. 204. *B. capitulata,* CBS 287.82. Conidiophores with terminal groups of primary conidia bearing secondary conidia, primary conidia with secondary conidia and setae, and sessile conidia on hyphae. MEYA, 22°C, 10 days.

61.4. *Blastobotrys elegans* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 3–5 mm in diameter, cerebriform, whitish and in later stages with snow-white, farinose patches. Budding cells are mostly absent; hyphae are hyaline, 2–3 µm wide. Conidia are formed on loose apical clusters of cylindroidal denticles, that are up to 3 µm long. Primary conidia are subglobose, (2.8–4.2) µm wide, without setae and densely crowded with globose, synchronously formed secondary conidia that are (1.8–3.4) µm in diameter (Fig. 205).

Fermentation: absent.

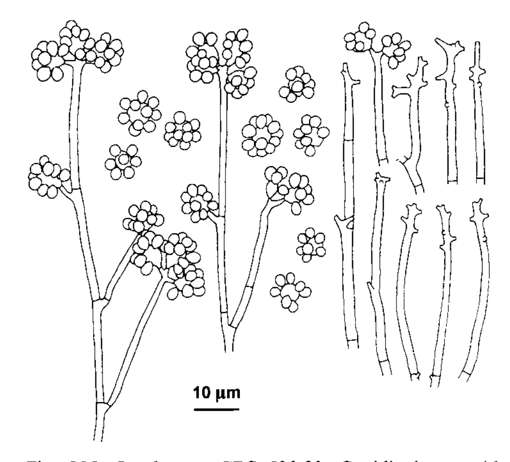


Fig. 205. *B. elegans,* CBS 530.83. Conidiophores with and without clusters of primary conidia which bear secondary conidia; denticles remain after detachment. MEYA, 22°C, 10 days.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	v	D-Gluconate	n
Soluble starch	v	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Growth at 37°C –
Co-Q: Not determined.
Mol% G + C: Not determined.

Origin of the strain studied: CBS 530.83, from house air, Rantio-Lehtimäki, Finland.
Type strain: CBS 530.83.

Comments: The species is recognizable by the absence of yeastlike growth and by a grape-like arrangement of conidia which do not easily separate.

61.5. *Blastobotrys gigas* de Hoog, Rantio-Lehtimäki & M.Th. Smith (1985)

See under teleomorph: *Stephanoascus farinosus* de Hoog, Rantio-Lehtimäki & M.Th. Smith: p. 401.

61.6. *Blastobotrys nivea* von Klopotek (1967)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 14 mm in diameter, dry, farinose, somewhat folded, snow-white and consist of compact mycelium; budding cells are absent. Hyphae are hyaline and (2–3) µm wide. Conidiophores are ascending, up to 230 µm high and the apical parts have distant geniculations with cylindrical denticles at the edges. Primary conidia are globose, (2.5–4.5) µm wide, without setae, and synchronously produce globose, densely crowded secondary conidia that are (2.0–2.5) µm in diameter (Fig. 206).

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	+		

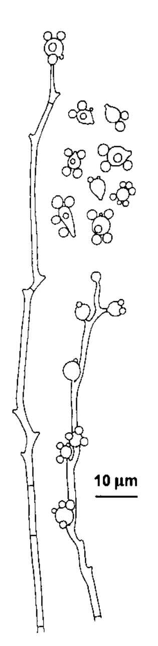


Fig. 206. *B. nivea*, CBS 163.67. Conidiophores with primary conidia bearing secondary conidia. MEYA, 22°C, 10 days.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	v	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	+
Inulin	v	D-Gluconate	n
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Growth at 37°C +
Co-Q: Not determined.
Mol% G + C: Not determined.

Origin of the strain studied: CBS 163.67 (ATCC 18420), from compost, Germany, von Klopotek.
Type strain: CBS 163.67.

Comments: Physiological characteristics are often difficult to judge because of formation of hyphal clumps. Morphologically, the species is recognized by slender conidiophores with a long geniculate rachis. Conidia are borne on widely spaced cylindroidal denticles.

61.7. *Blastobotrys proliferans* Marvanová (1976)

Synonym:
Blastobotrys navarrensis Sesma & C. Ramírez (1978)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, the colonies are 10 mm in diameter, dry, thinly hairy, soft and snow-white; budding cells are absent. Hyphae are hyaline, and (1.5–2.5) µm wide. Conidiophores are ascending, up to 80 µm long, and barely different from the remaining hyphae; apically, they produce 1–3 pedicels up to 5 µm long bearing conidia. Primary conidia are pear-shaped, about (3.0–4.5)×(4.5–8.0) µm, contain a refractive granular body, and bear a seta which again produces 1–3 similar conidia at its apex. The primary conidia synchronously produce densely crowded globose secondary conidia that are 1.5–2.5 µm in diameter (Fig. 207).

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	v
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	v	D-Gluconate	n
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	v	Hexadecane	n
L-Rhamnose	v	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

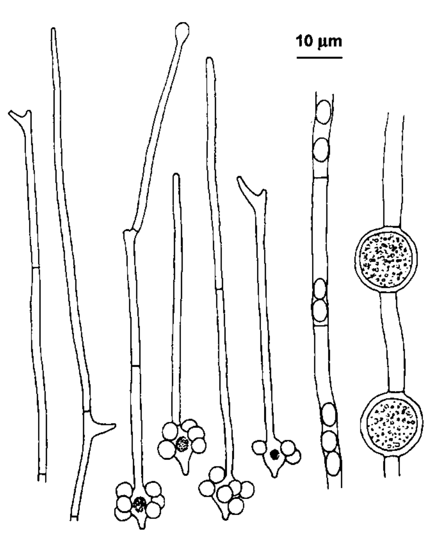


Fig. 207. *B. proliferans*, CBS 522.75. Conidiophores and primary conidia bearing secondary conidia and setae, endoconidia in hyphae, and intercalary chlamydospores. MEYA, 22°C, 10 days.

Additional assimilation tests and other growth characteristics:

Growth at 37°C +

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strains studied: CBS 522.75, from a mite-infested nut, Brazil; CBS 139.77, type strain of *B. navarrensis,* from black pepper, Spain; from soil (1); from man (1); from house dust (1); from linoleum (1).

Type strain: CBS 522.75.

Comments: Key features are the primary conidia which contain granular, highly refractive bodies and the setae which bear new, similar conidia.

Comments on the genus

Budding cells are usually absent or in low abundance when present. Until recently, the genus was treated as a hyphomycete, close to the ascomycetous genus *Sporothrix* Hektoen & Perkins, from which it was distinguished on morphological criteria. However, members of *Blastobotrys,* as well as some *Sporothrix* species, were shown to have micropores in their septa (Smith and Batenburg-van der Vegte 1985, 1986b), coenzyme Q-9 systems (Suzuki and Nakase 1986, Yamada and Smith 1985), and occasionally a *Stephanoascus* teleomorph (Smith et al. 1981, de Hoog et al. 1985). Consequently, *Blastobotrys* is maintained here for saccharomycetous fungi and treated under the yeasts, while *Sporothrix* should be reserved for anamorphs of the Ophiostomatales (de Hoog 1992).

Species delimitation in the genus has not yet been satisfactorily established. Most species are known from a single strain. The taxonomic value of morphological and physiological criteria has to be evaluated on the basis of teleomorphs and genomic data. To this end, Kurtzman and Robnett (1995) showed from nucleotide sequence analysis of 5′ end large subunit rDNA that species of *Blastobotrys* are members of a clade that includes *Arxula, Sympodiomyces* and *Stephanoascus* (Fig. 170, p. 385).

62. *Botryozyma* Shann & M.Th. Smith

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is by multilateral budding. Cells are cylindrical. Pseudohyphae are formed. Terminal pseudohyphal cells are transformed into simple or complex appressoria.

Ascospores are not formed.

Sugars are not fermented. Nitrate is not assimilated. Urease is not produced. Diazonium blue B reaction is negative.

Type species

Botryozyma nematodophila Shann & M.Th. Smith

Species accepted

Botryozyma nematodophila Shann & M.Th. Smith (1992)

Systematic discussion of the species

62.1. *Botryozyma nematodophila* Shann & M.Th. Smith (Smith et al. 1992)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are cylindrical, (8.0–22.5)×(2.0–5.5) µm, reproduce predominantly in the apical zone and occur in pairs and short chains, but mainly in pseudomycelial elements; fusiform cells may be present. End cells are usually transformed into simple or complex branch-like structures (appressoria), (2.0–20.0)×(1.0–2.0) µm (Fig. 208). A sediment is formed.

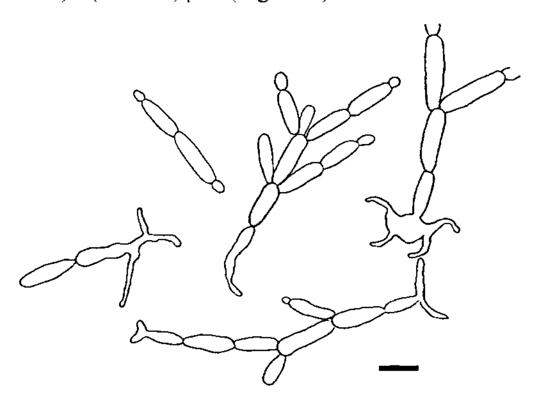


Fig. 208. *B. nematodophila,* CBS 7426. Budding cells, pseudohyphae and appressoria in glucose–peptone–yeast extract after 3 days at 25°C. Bar = 5 µm.

Growth on glucose–yeast extract–peptone agar: After 3 days at 25°C, the cells are similar in shape and size as in glucose–yeast extract–peptone water. Appressorial cells are present. The streak culture is cream-colored, dull, delicately wrinkled, butyrous, and the margin slightly lobed.

Dalmau plate cultures on glucose–yeast extract–peptone agar: After 3 days at 25°C, a well-developed pseudomycelium is formed. Fusiform cells may be present, and simple as well as complex appressoria are formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	–	Growth at 37°C	+
0.1% Cycloheximide	+		

Co-Q: Not determined.

Mol% G + C: 29.5, CBS 7426; 29.7, CBS 7442 (T_m: Smith et al. 1992).

Origin of the strains studied: CBS 7426, CBS 7442, from *Panagrellus zymosiphilus* in sour rot of grapes, Italy, C. Shann.

Type strain: CBS 7426, isolated by C. Shann.

Comments on the genus

The monotypic genus *Botryozyma* was introduced by Smith et al. (1992) to accommodate two yeast isolates found along with nematodes in and on damaged grapes. According to Shann (1988), the species is a saprophyte on the nematode *Panagrellus zymosiphilus,* to which it may be attached by appressoria.

63. *Brettanomyces* Kufferath & van Laer

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is either by multilateral budding or, rarely, by bipolar budding in basipetal succession on a narrow base. Cells are either spheroidal, subglobose to ellipsoidal, frequently ogival, or cylindroidal to elongate. Pseudomycelium is simple or well-developed, and branched. One-celled, non-septate mycelium may be formed.

Cultures grow slowly and are usually short-lived. Acetic acid is produced aerobically from glucose. Fermentation is usually stimulated by molecular oxygen. An extraneous vitamin source is required. Diazonium blue B reaction is negative.

The teleomorphic genus is *Dekkera.*

Type species

Brettanomyces bruxellensis Kufferath & van Laer

Species accepted

1. *Brettanomyces anomalus* Custers (1940): see *Dekkera anomala*
2. *Brettanomyces bruxellensis* Kufferath & van Laer (1921): see *Dekkera bruxellensis*
3. *Brettanomyces custersianus* van der Walt (1961)
4. *Brettanomyces naardenensis* Kolfschoten & Yarrow (1970)
5. *Brettanomyces nanus* (M.Th. Smith, Batenburg-van der Vegte & Scheffers) M.Th. Smith, Boekhout, Kurtzman & O'Donnell (1994)

Key to species

See Table 49.

1. a D-Glucitol assimilated → 2
   b D-Glucitol not assimilated → 3

2(1). a Succinate assimilated . . . . *B. naardenensis*: p. 451
   b Succinate not assimilated . . . . *B. nanus*: p. 452

3(1). Species with the following combinations of characters:
   a Slender, often branched, non-septate filaments are produced on dalmau plates; lactose fermented by most strains; succinate latently assimilated by most strains; galactose assimilated . . . . *Dekkera anomala*: p. 174
   b Non-septate filaments are not produced; lactose not fermented; succinate not assimilated; galactose assimilation variable . . . . *D. bruxellensis*: p. 175
   c Non-septate filaments are not produced; lactose not fermented; succinate assimilated; galactose not assimilated . . . . *B. custersianus*: p. 451

Table 49
Key characters of species in the genus *Brettanomyces* and its teleomorph *Dekkera*

Species	Lactose fermentation	Assimilation			Non-septate filaments
		Galactose	Glucitol	Succinate	
Dekkera anomala	v	+	–	v	+
D. bruxellensis	–	v	–	–	–
Brettanomyces custersianus	–	–	–	+	–
B. naardenensis	–	+	+	+	–
B. nanus	–	+	+	–	–

Systematic discussion of the species

63.1. *Brettanomyces anomalus* Custers (1940)

See teleomorph: *Dekkera anomala* M.Th. Smith & van Grinsven: p. 174.

63.2. *Brettanomyces bruxellensis* Kufferath & van Laer (1921)

See teleomorph: *Dekkera bruxellensis* van der Walt: p. 175.

63.3. *Brettanomyces custersianus* van der Walt (1961)

Synonym:
Dekkera custersiana F.-L. Lee & S.-C. Jong (1986a)

Growth in malt extract: After 5 days at 25°C, the cells are ellipsoidal, frequently ogival and cylindroidal to elongate, (1.5–3.5)×(2.0–19.0) µm. Cells reproduce by budding, and occur singly, in pairs or in short, occasionally somewhat branched chains. Pseudomycelium is produced. A floccose sediment is formed. A thin, dull pellicle or a slight, frequently incomplete ring is sometimes present.

Growth on malt agar + 2% calcium carbonate: After 6 weeks at room temperature, the streak culture is raised, cream-colored to light brown, shiny to dull, verrucose, and crispulate or rugose. The margin is undulating to lobiform. Acetic acid production is slight.

Dalmau plate cultures on potato-, rice- and morphology agars: Pseudomycelium is abundantly produced and consists of filamentous cells with few or no blastospores. Blastospores are more abundant under anaerobic conditions, and are arranged in short chains or weakly branched verticils (Fig. 209).

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	–		

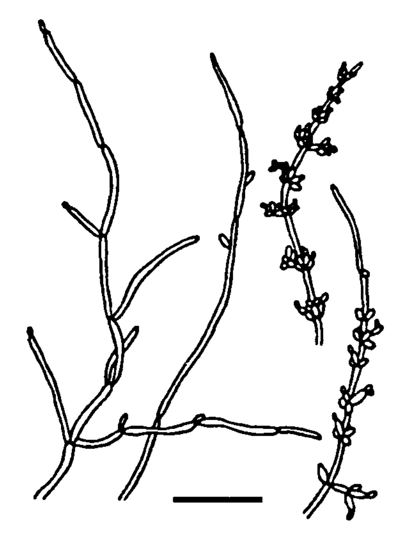


Fig. 209. *B. custersianus*, CBS 4805. Pseudomycelium on corn meal agar after 10 days at 25°C (from van der Walt 1970e). Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	–	Growth at 35°C	+
0.01% Cycloheximide	+	Growth at 37°C	+
0.1% Cycloheximide	s		

Co-Q: 9 (Yamada et al. 1980, Billon-Grand 1987).
Mol% G + C: 38.9–39.3, 4 strains, CBS 4805, CBS 4806, CBS 5207, CBS 5208 (T_m: Smith et al. 1990b).

Origin of the strain studied: CBS 4805, CBS 4806, CBS 5207, CBS 5208, equipment at a Bantu beer brewery, South Africa, van der Walt.

Type strain: CBS 4805 (ATCC 34446), isolated by van der Walt.

63.4. *Brettanomyces naardenensis* Kolfschoten & Yarrow (1970)

Synonym:
Dekkera naardenensis S.-C. Jong & F.-L. Lee (1986)

Growth in malt extract: After 5 days at 25°C, the cells are predominantly cylindroidal to elongate, rarely ellipsoidal or ogival, (1.5–4.5)×(4.0–15.0–30) µm, and reproduce by budding. They occur singly, in pairs or in short chains or small clusters. Pseudomycelium is abundant. Floccose sediment is formed; occasionally a slight, incomplete ring and islets are present.

Growth on malt agar + 2% calcium carbonate: After 6 weeks at room temperature, the streak culture is rather coherent, creamish-brown to light brown, somewhat shiny to rather dull, and pulvinate to verruculose. The margin is lobate. Acetic acid production is not very pronounced.

Dalmau plate cultures on potato-, rice- and morphology agars: A ramified pseudomycelium is produced that bears few or no blastospores. Pseudomycelium is better developed under anaerobic conditions.

Fermentation:

Glucose	s	Lactose	–
Galactose	w/–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	v	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	–	0.1% Cycloheximide	+
0.01% Cycloheximide	+	Growth at 35°C	–

Co-Q: 9 (Yamada et al. 1980, Billon-Grand 1987).
Mol% G+C: 42.2–43.3, 3 strains, CBS 6042, CBS 6107, CBS 6117 (T_m: Smith et al. 1990b).

Origin of the strains studied: CBS 6042, lemonade, Netherlands, G.A. Kolfschoten; CBS 6040, soda water, U.S.A., D.G. Ahearn; the following were isolated by G.A. Kolfschoten: CBS 6041, carbonated lemonade pH 2.7, Belgium; CBS 6043, carbonated tonic water pH 3, Netherlands; CBS 6107, carbonated lemonade pH 3.1, Norway; CBS 6115, carbonated soft drink pH 2.7, Netherlands; CBS 6117, carbonated lemonade pH 3, Denmark; CBS 6118, carbonated lemonade, Netherlands.

Type strain: CBS 6042 (ATCC 22075).

63.5. *Brettanomyces nanus* (M.Th. Smith, Batenburg-van der Vegte & Scheffers) M.Th. Smith, Boekhout, Kurtzman & O'Donnell (Boekhout et al. 1994)

Synonyms:
Brettanomyces nanus Scheffers (1966) nom. inval.
Eeniella nana M.Th. Smith, Batenburg-van der Vegte & Scheffers (1981)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are usually ogival to apiculate, (1.8–4.5)×(2.2–7.5) μm, seldom spheroidal or cylindroidal, single, or in pairs. Pear-shaped cells are sometimes present (Fig. 210). After 1 month, sediment is formed.

Growth on malt agar + 2% calcium carbonate: After 1 month, the streak culture is white to cream-colored, smooth, and glossy; in cross-section, the colony shows a slightly raised center and a flat periphery.

Dalmau plate cultures on potato-, rice- and corn meal agars: Simple pseudohyphae consisting of short chains of cells are occasionally present.

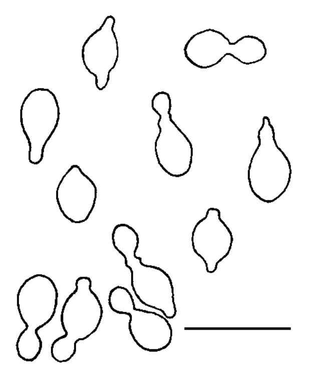


Fig. 210. *B. nanus*, CBS 1956. Pyriform cells in glucose–yeast extract–peptone water after 3 days at 25°C. Bar = 10 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Starch formation	–	0.1% Cycloheximide	–
0.01% Cycloheximide	+	Growth at 35°C	–

Co-Q: 9 (Yamada et al. 1980).
Mol% G+C: 40.2–41.4, 3 strains, CBS 1945, CBS 1955, CBS 1956 (T_m: Smith et al. 1981).

Origin of the strains studied: CBS 1945, bottled beer, brewery, Kalmar, Sweden, G. Een; CBS 1955, bottled beer, brewery, Stockholm, Sweden, G. Een; CBS 1956, bottled beer, brewery, Göteborg, Sweden, G. Een.

Type strain: CBS 1945 (ATCC 48014).

Comments: *Brettanomyces nanus* was introduced by Scheffers (1966) without a Latin diagnosis. Smith et al. (1981) examined the three available isolates and found bipolar budding. On the basis of this morphological feature and the physiological property of acetic acid production, Smith et al. (1981) considered it incorrect to classify these isolates in *Brettanomyces* or in any other extant genus with bipolar budding. Therefore, they

introduced the genus *Eeniella* to accommodate these apiculate, acetic acid-producing strains. However, on the basis of partial 26S rDNA sequence data, Boekhout et al. (1994) considered *Eeniella* a synonym of *Brettanomyces* and consequently reintroduced and validated the species *B. nanus.*

Comments on the genus

For a detailed historical account of the genus *Brettanomyces,* see Custers (1940) and van der Walt (1970e, 1984c). Fiol and Billon-Grand (1978b) studied the production of intracellular oxidases, nitrite and nitrate reductases in *Brettanomyces* and *Dekkera* and discussed the relationship between the two genera. The coenzyme Q systems were studied by Yamada et al. (1980) and Billon-Grand (1987); the latter author stressed the importance of minor ubiquinones in the classification of both genera. In addition to the major component Co-Q 9, Co-Q 8 was found as a minor component in all *Brettanomyces* and *Dekkera* species. Traces of Co-Q 7 and Co-Q 6 were found in a few species. A series of publications appeared on ascospore formation in type strains of five *Brettanomyces* species (Lee and Jong 1985, 1986a,b, Jong and Lee 1986); as a consequence, these species were reclassified in the teleomorphic genus *Dekkera.* The presence of ascospores was demonstrated by staining. However, Smith et al. (1990b) considered the hat-shaped spores to be artifacts that may have resulted from contraction of cellular cytoplasm during staining. Therefore, Smith et al. (1990b) did not accept the species *D. custersianus* and *D. naardenensis.* On the basis of DNA reassociations and enzyme comparisons, Smith et al. (1990b) accepted four species in *Brettanomyces,* two of which lack a teleomorphic state. Various studies of mtDNA (McArthur and Clark-Walker 1983, Hoeben and Clark-Walker 1986, Clark-Walker et al. 1987, Hoeben et al. 1993), rRNA (Molina et al. 1993), rDNA (Boekhout et al. 1994) and rRNA (Yamada et al. 1994d) have been performed on *Brettanomyces* and the teleomorph *Dekkera* (see *Dekkera,* Comments on the genus).

64. *Candida* Berkhout

S.A. Meyer, R.W. Payne and D. Yarrow

Diagnosis of the genus

Cells are globose, ellipsoidal, cylindroidal, or elongate, occasionally ogival, triangular or lunate. Reproduction is by holoblastic budding. Pseudohyphae and septate hyphae may be formed. The cell wall is ascomycetous and two-layered. Arthroconidia and ballistoconidia are not formed. Starch-like compounds are not produced. Diazonium blue B reaction is negative. Xylose, rhamnose and fucose are not present in cell hydrolyzates.

Type species

Candida vulgaris Berkhout [synonym of *Candida tropicalis* (Castellani) Berkhout]

Species accepted

1. *Candida aaseri* Dietrichson ex van Uden & H.R. Buckley (1970)
2. *Candida albicans* (Robin) Berkhout (1923)
3. *Candida amapae* Morais, Rosa, S.A. Meyer, Mendonça-Hagler & Hagler (1995)
4. *Candida anatomiae* (Zwillenberg) S.A. Meyer & Yarrow 1978
5. *Candida ancudensis* C. Ramírez & A. González (1984)
6. *Candida antillancae* C. Ramírez & A. González (1984)
7. *Candida apicola* (Hajsig) S.A. Meyer & Yarrow (1978)
8. *Candida apis* (Lavie ex van Uden & Vidal-Leiria) S.A. Meyer & Yarrow (1978)
9. *Candida atlantica* (Siepmann) S.A. Meyer & Simione ex S.A. Meyer & Yarrow (1998)
10. *Candida atmosphaerica* Santa María (1959)
11. *Candida auringiensis* Santa María (1978)
12. *Candida austromarina* (Fell & I.L. Hunter) S.A. Meyer & Yarrow (1978)
13. *Candida azyma* (van der Walt, E. Johannsen & Yarrow) S.A. Meyer & Yarrow (1978)
14. *Candida beechii* H.R. Buckley & van Uden (1968)
15. *Candida bertae* C. Ramírez & A. González (1984)
16. *Candida berthetii* Boidin, Pignal, Mermier & Arpin (1963)
17. *Candida blankii* H.R. Buckley & van Uden (1968)
18. *Candida boidinii* C. Ramírez (1953)
19. *Candida boleticola* Nakase (1971)
20. *Candida bombi* Montrocher (1967)
21. *Candida bombicola* (J.F.T. Spencer, Gorin & Tulloch) S.A. Meyer & Yarrow (1978)
22. *Candida buinensis* Soneda & S. Uchida (1971)
23. *Candida butyri* Nakase (1971)
24. *Candida cantarellii* (van der Walt & van Kerken) S.A. Meyer & Yarrow (1978)
25. *Candida caseinolytica* Phaff, Starmer, Lachance & Ganter (1994)
26. *Candida castellii* (Capriotti) S.A. Meyer & Yarrow (1978)
27. *Candida castrensis* C. Ramírez & A. González (1984)
28. *Candida catenulata* Diddens & Lodder (1942)
29. *Candida chilensis* Grinbergs & Yarrow (1970)
30. *Candida chiropterorum* Grose & Marinkelle (1968)
31. *Candida coipomoensis* C. Ramírez & A. González (1984)
32. *Candida conglobata* (Redaelli) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)
33. *Candida cylindracea* K. Yamada & Machida ex S.A. Meyer & Yarrow (1998)
34. *Candida dendrica* (van der Walt, van der Klift & D.B. Scott) S.A. Meyer & Yarrow (1978)
35. *Candida dendronema* van der Walt, van der Klift & D.B. Scott (1971)
36. *Candida diddensiae* (Phaff, Mrak & Williams) Fell & S.A. Meyer ex S.A. Meyer & Ahearn (1983)
37. *Candida diversa* Y. Ohara, Nonomura & Yunome ex van Uden & H.R. Buckley (1970)
38. *Candida drimydis* C. Ramírez & A. González (1984)
39. *Candida edax* van der Walt & E.E. Nel (1968): see *Stephanoascus smithiae*, p. 402
40. *Candida entomophila* D.B. Scott, van der Walt & van der Klift (1971)
41. *Candida ergastensis* Santa María (1971)

42. *Candida ernobii* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (1978)
43. *Candida etchellsii* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (1978)
44. *Candida ethanolica* Rybářová, Štros & Kocková-Kratochvílová (1980)
45. *Candida famata* (F.C. Harrison) S.A. Meyer & Yarrow (1978)
    a. *Candida famata* (F.C. Harrison) S.A. Meyer & Yarrow var. *famata* (1985): see *Debaryomyces hansenii* var. *hansenii,* p. 161
    b. *Candida famata* var. *flareri* (Ciferri & Redaelli) Nakase & M. Suzuki (1985): see *Debaryomyces hansenii* var. *fabryi,* p. 162
46. *Candida fennica* (Sonck & Yarrow) S.A. Meyer & Ahearn (1983)
47. *Candida fermenticarens* van der Walt & P.B. Baker (1978)
48. *Candida floricola* Tokuoka, Ishitani, S. Goto & Komagata (1987)
49. *Candida fluviatilis* Hedrick (1976)
50. *Candida freyschussii* H.R. Buckley & van Uden (1968)
51. *Candida friedrichii* van Uden & Windisch (1968)
52. *Candida fructus* (Nakase) S.A. Meyer & Yarrow (1978)
53. *Candida galacta* (Golubev & Bab'eva) F.-L. Lee, C.-F. Lee, Okada, Komagata & Kozak (1993)
54. *Candida geochares* (van der Walt, E. Johannsen & Yarrow) S.A. Meyer & Yarrow (1978)
55. *Candida glabrata* (H.W. Anderson) S.A. Meyer & Yarrow (1978)
56. *Candida glaebosa* Komagata & Nakase (1965)
57. *Candida glucosophila* Tokuoka, Ishitani, S. Goto & Komagata (1987)
58. *Candida gropengiesseri* (F.C. Harrison) S.A. Meyer & Yarrow (1978)
59. *Candida guilliermondii* (Castellani) Berkhout (1923)
    a. *Candida guilliermondii* (Castellani) Berkhout var. *guilliermondii* (1952): see *Pichia guilliermondii,* p. 308
    b. *Candida guilliermondii* var. *membranifaciens* Lodder & Kreger-van Rij (1952): see *Pichia ohmeri,* p. 329
60. *Candida haemulonii* (van Uden & Kolipinski) S.A. Meyer & Yarrow (1978)
61. *Candida homilentoma* van der Walt & Nakase (1973)
62. *Candida humilis* (E.E. Nel & van der Walt) S.A. Meyer & Yarrow (1978)
63. *Candida incommunis* Y. Ohara, Nonomura & T. Yamazaki (1965)
64. *Candida inconspicua* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (1978)
65. *Candida insectalens* (D.B. Scott, van der Walt & van der Klift) S.A. Meyer & Yarrow (1978)
66. *Candida insectamans* D.B. Scott, van der Walt & van der Klift (1972)
67. *Candida insectorum* D.B. Scott, van der Walt & van der Klift (1972)
68. *Candida intermedia* (Ciferri & Ashford) Langeron & Guerra (1938)
69. *Candida ishiwadae* Sugiyama & S. Goto (1969)
70. *Candida karawaiewii* Yarrow & S.A. Meyer (1978)
71. *Candida krissii* S. Goto & Iizuka (1974)
72. *Candida kruisii* (Kocková-Kratochvílová & Ondrušová) S.A. Meyer & Yarrow (1978)
73. *Candida krusei* (Castellani) Berkhout (1923): see *Issatchenkia orientalis,* p. 222
74. *Candida lactis-condensi* (B.W. Hammer) S.A. Meyer & Yarrow (1978)
75. *Candida laureliae* C. Ramírez & A. González (1984)
76. *Candida lipolytica* (F.C. Harrison) Diddens & Lodder (1942): see *Yarrowia lipolytica,* p. 420
77. *Candida llanquihuensis* C. Ramírez & A. González (1984)
78. *Candida lusitaniae* van Uden & do Carmo-Sousa (1959): see *Clavispora lusitaniae,* p. 148
79. *Candida lyxosophila* van der Walt, N.P. Ferreira & Steyn (1987)
80. *Candida magnoliae* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (1978)
81. *Candida maltosa* Komagata, Nakase & Katsuya (1964)
82. *Candida maris* (van Uden & Zobell) S.A. Meyer & Yarrow (1978)
83. *Candida maritima* (Siepmann) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)
84. *Candida melibiosica* H.R. Buckley & van Uden (1968)
85. *Candida membranifaciens* (Lodder & Kreger-van Rij) Wickerham & K.A. Burton (1954)
86. *Candida mesenterica* (A. Geiger) Diddens & Lodder (1942)
87. *Candida methanosorbosa* (Abe & Yokote) J.A. Barnett, R.W. Payne & Yarrow (1983)
88. *Candida milleri* Yarrow (1978)
89. *Candida mogii* Vidal-Leiria (1967)
90. *Candida montana* S. Goto & Oguri (1983)
91. *Candida multigemmis* (Buhagiar) S.A. Meyer & Yarrow (1978)

92. *Candida musae* (Nakase) S.A. Meyer & Yarrow (1978)
93. *Candida naeodendra* van der Walt, E. Johannsen & Nakase (1973)
94. *Candida nanaspora* Saëz & Rodrigues de Miranda (1988)
95. *Candida natalensis* van der Walt & Tscheuschner (1957)
96. *Candida nemodendra* (van der Walt, van der Klift & D.B. Scott) S.A. Meyer & Yarrow (1978)
97. *Candida nitratophila* (Shifrine & Phaff) S.A. Meyer & Yarrow (1978)
98. *Candida norvegica* (Reiersöl) S.A. Meyer & Yarrow (1978)
99. *Candida odintsovae* Bab'eva, Reshetova, Blagodatskaya & Galimova (1989)
100. *Candida oleophila* Montrocher (1967)
101. *Candida oregonensis* Phaff & do Carmo-Sousa (1962)
102. *Candida ovalis* Kumamoto & Yamamoto (1986)
103. *Candida palmioleophila* Nakase & M. Itoh (1988)
104. *Candida paludigena* Golubev & Blagodatskaya (1981)
105. *Candida parapsilosis* (Ashford) Langeron & Talice (1932)
106. *Candida pararugosa* Nakase, Komagata & Fukazawa (1978)
107. *Candida peltata* (Yarrow) S.A. Meyer & Ahearn (1983)
108. *Candida petrohuensis* C. Ramírez & A. González (1984)
109. *Candida pignaliae* (F.H. Jacob) S.A. Meyer & Yarrow (1978)
110. *Candida pini* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (1978)
111. *Candida populi* Hagler, Mendonça-Hagler & Phaff (1989)
112. *Candida pseudointermedia* Nakase, Komagata & Fukazawa (1976)
113. *Candida pseudolambica* M.Th. Smith, Poot & Kull (1989)
114. *Candida psychrophila* (S. Goto, Sugiyama & Iizuka) S.A. Meyer & Yarrow (1978)
115. *Candida pulcherrima* (Lindner) Windisch (1940): see *Metschnikowia pulcherrima,* p. 264
116. *Candida quercitrusa* (van Uden & do Carmo-Sousa) S.A. Meyer & Phaff ex S.A. Meyer & Yarrow (1998)
117. *Candida quercuum* Nakase (1971)
118. *Candida railenensis* C. Ramírez & A. González (1984)
119. *Candida reukaufii* (Grüss) Diddens & Lodder (1942): see *Metschnikowia reukaufii,* p. 265
120. *Candida rhagii* Jurzitza, Kühlwein & Kreger-van Rij (1960)
121. *Candida rugopelliculosa* Nakase (1971)
122. *Candida rugosa* (H.W. Anderson) Diddens & Lodder (1942)
123. *Candida saitoana* Nakase & M. Suzuki (1985)
124. *Candida sake* (Saito & Oda) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)
125. *Candida salmanticensis* (Santa María) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)
126. *Candida santamariae* Montrocher (1967)
127. *Candida santjacobensis* C. Ramírez & A. González (1984)
128. *Candida savonica* Sonck (1974)
129. *Candida schatavii* (Kocková-Kratochvílová & Ondrušová) S.A. Meyer & Yarrow (1978)
130. *Candida sequanensis* Saëz & Rodrigues de Miranda (1984)
131. *Candida shehatae* H.R. Buckley & van Uden (1967)
    a. *Candida shehatae* H.R. Buckley & van Uden var. *shehatae* (1990)
    b. *Candida shehatae* var. *insectosa* Kurtzman (1990)
    c. *Candida shehatae* var. *lignosa* Kurtzman (1990)
132. *Candida silvae* Vidal-Leiria & van Uden (1963)
133. *Candida silvanorum* van der Walt, van der Klift & D.B. Scott (1971)
134. *Candida silvatica* (van der Walt, van der Klift & D.B. Scott) S.A. Meyer & Yarrow (1978)
135. *Candida silvicultrix* van der Walt, D.B. Scott & van der Klift (1972)
136. *Candida solani* Lodder & Kreger-van Rij (1952)
137. *Candida sonorensis* (M.W. Miller, Phaff, Miranda, Heed & Starmer) S.A. Meyer & Yarrow (1978)
138. *Candida sophiae-reginae* C. Ramírez & A. González (1984)
139. *Candida sorbophila* (Nakase) S.A. Meyer & Yarrow (1978)
140. *Candida sorboxylosa* Nakase (1971)
141. *Candida spandovensis* (Henninger & Windisch) S.A. Meyer & Yarrow (1978)
142. *Candida stellata* (Kroemer & Krumbholz) S.A. Meyer & Yarrow (1978)
143. *Candida succiphila* J.-D. Lee & Komagata (1980)
144. *Candida suecica* Rodrigues de Miranda & Norkrans (1968)

145. *Candida tanzawaensis* Nakase & M. Itoh (1988)
146. *Candida tenuis* Diddens & Lodder (1942)
147. *Candida tepae* Grinbergs (1967)
148. *Candida torresii* (van Uden & Zobell) S.A. Meyer & Yarrow (1978)
149. *Candida tropicalis* (Castellani) Berkhout (1923)
150. *Candida tsuchiyae* Nakase & M. Suzuki (1985)
151. *Candida utilis* (Henneberg) Lodder & Kreger-van Rij (1952): see *Pichia jadinii*, p. 314
152. *Candida vaccinii* Tokuoka, Ishitani, S. Goto & Komagata (1987)
153. *Candida valdiviana* Grinbergs & Yarrow (1970)
154. *Candida valida* (Leberle) van Uden & H.R. Buckley (1970): see *Pichia membranifaciens*, p. 319
155. *Candida vanderwaltii* (Vidal-Leiria) S.A. Meyer & Yarrow (1978)
156. *Candida vartiovaarae* (Capriotti) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)
157. *Candida versatilis* (Etchells & T.A. Bell) S.A. Meyer & Yarrow (1978)
158. *Candida vinaria* Y. Ohara, Nonomura & Yunome ex M.Th. Smith (1973)
159. *Candida vini* (Vallot ex Desmazières) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)
160. *Candida viswanathii* Viswanathan & H.S. Randhawa ex R.S. Sandhu & H.S. Randhawa (1962)
161. *Candida wickerhamii* (Capriotti) S.A. Meyer & Yarrow (1978)
162. *Candida xestobii* Yarrow & S.A. Meyer (1978)
163. *Candida zeylanoides* (Castellani) Langeron & Guerra (1938)

Key to species

It must be noted that *C. famata* is not included in the key because it is variable in many of the tests.

1. a Nitrate growth positive → 2
b Nitrate growth negative → 29
2(1). a Melezitose growth positive → 3
b Melezitose growth negative → 13
3(2). a Raffinose growth positive → 4
b Raffinose growth negative → 7
4(3). a Glucose fermentation positive → 5
b Glucose fermentation negative → 6
5(4). a Galactose growth positive ... *C. valdiviana*: p. 566
b Galactose growth negative ... *C. utilis*: p. 565
6(4). a Growth at 37°C positive ... *C. edax*: p. 500
b Growth at 37°C negative ... *C. bertae*: p. 486
7(3). a Galactose growth positive → 8
b Galactose growth negative → 10
8(7). a Soluble starch growth positive ... *C. ishiwadae*: p. 516
b Soluble starch growth negative → 9
9(8). a Lactose growth positive ... *C. chilensis*: p. 495
b Lactose growth negative ... *C. incommunis*: p. 513
10(7). a Soluble starch growth positive ... *C. ishiwadae*: p. 516
b Soluble starch growth negative → 11
11(10). a L-Rhamnose growth positive ... *C. populi*: p. 541
b L-Rhamnose growth negative → 12
12(11). a Erythritol growth positive ... *C. incommunis*: p. 513
b Erythritol growth negative ... *C. vartiovaarae*: p. 567
13(2). a Erythritol growth positive → 14
b Erythritol growth negative → 15
14(13). a Galactose growth positive ... *C. nanaspora*: p. 530
b Galactose growth negative ... *C. boidinii*: p. 488
15(13). a Growth at 37°C positive → 16
b Growth at 37°C negative → 20
16(15). a Galactose growth positive → 17
b Galactose growth negative → 19
17(16). a L-Rhamnose growth positive ... *C. methanosorbosa*: p. 526
b L-Rhamnose growth negative → 18
18(17). a Hexadecane growth positive ... *C. vaccinii*: p. 565
b Hexadecane growth negative ... *C. magnoliae*: p. 521
19(16). a Cellobiose growth positive ... *C. berthetii*: p. 487
b Cellobiose growth negative ... *C. glucosophila*: p. 510
20(15). a Cellobiose growth positive → 21
b Cellobiose growth negative → 24
21(20). a Galactose growth positive → 22
b Galactose growth negative → 23

22(21). a L-Rhamnose growth positive .... *C. wickerhamii*: p. 570
b L-Rhamnose growth negative .... *C. versatilis*: p. 567
23(21). a Soluble starch growth positive .... *C. ernobii*: p. 501
b Soluble starch growth negative .... *C. norvegica*: p. 532
24(20). a Raffinose growth positive .... *C. lactis-condensi*: p. 519
b Raffinose growth negative → 25
25(24). a L-Rhamnose growth positive .... *C. nitratophila*: p. 531
b L-Rhamnose growth negative → 26
26(25). a Glucose fermentation positive → 27
b Glucose fermentation negative → 28
27(26). a 0.01% Cycloheximide growth positive .... *C. pignaliae*: p. 539
b 0.01% Cycloheximide growth negative .... *C. etchellsii*: p. 502
28(26). a 0.01% Cycloheximide growth positive .... *C. vanderwaltii*: p. 566
b 0.01% Cycloheximide growth negative .... *C. etchellsii*: p. 502
29(1). a Erythritol growth positive → 30
b Erythritol growth negative → 86
30(29). a 0.01% Cycloheximide growth positive → 31
b 0.01% Cycloheximide growth negative → 64
31(30). a Soluble starch growth positive → 32
b Soluble starch growth negative → 38
32(31). a Growth at 37°C positive → 33
b Growth at 37°C negative → 36
33(32). a Lactose growth positive .... *C. blankii*: p. 487
b Lactose growth negative → 34
34(33). a Glucose fermentation positive → 35
b Glucose fermentation negative .... *C. chiropterorum*: p. 495
35(34). a Growth at 40°C positive .... *C. peltata*: p. 538
b Growth at 40°C negative .... *C. homilentoma*: p. 512
36(32). a L-Rhamnose growth positive .... *C. tenuis*: p. 561
b L-Rhamnose growth negative → 37
37(36). a DL-Lactate growth positive .... *C. shehatae* var. *lignosa*: p. 552
b DL-Lactate growth negative .... *C. shehatae* var. *shehatae*: p. 552
38(31). a Glucose fermentation positive → 39
b Glucose fermentation negative → 59
39(38). a Raffinose growth positive → 40
b Raffinose growth negative → 43
40(39). a Growth at 37°C positive → 41
b Growth at 37°C negative → 42
41(40). a L-Rhamnose growth positive .... *C. insectorum*: p. 515
b L-Rhamnose growth negative .... *C. entomophila*: p. 500
42(40). a Lactose growth positive .... *C. tenuis*: p. 561
b Lactose growth negative .... *C. santjacobensis*: p. 550
43(39). a Lactose growth positive → 44
b Lactose growth negative → 48
44(43). a Growth at 37°C positive .... *C. auringiensis*: p. 484
b Growth at 37°C negative → 45
45(44). a Melezitose growth positive → 46
b Melezitose growth negative → 47
46(45). a L-Rhamnose growth positive .... *C. tenuis*: p. 561
b L-Rhamnose growth negative .... *C. coipomoensis*: p. 496
47(45). a Trehalose fermentation positive .... *C. coipomoensis*: p. 496
b Trehalose fermentation negative .... *C. ergastensis*: p. 501
48(43). a L-Rhamnose growth positive → 49
b L-Rhamnose growth negative → 50
49(48). a Galactose growth positive .... *C. succiphila*: p. 559
b Galactose growth negative .... *C. boidinii*: p. 488
50(48). a Galactose growth positive → 51
b Galactose growth negative → 54
51(50). a Trehalose fermentation positive → 52
b Trehalose fermentation negative .... *C. cantarellii*: p. 492
52(51). a Galactose fermention positive .... *C. schatavii*: p. 551
b Galactose fermention negative → 53
53(52). a Pellicle formation positive .... *C. boleticola*: p. 489
b Pellicle formation negative .... *C. laureliae*: p. 519
54(50). a Cellobiose growth positive .... *C. ovalis*: p. 535
b Cellobiose growth negative → 55

55(54).	a	DL-Lactate growth positive	→ 56	
	b	DL-Lactate growth negative	→ 58	
56(55).	a	Trehalose fermention positive	*C. llanquihuensis*:	p. 520
	b	Trehalose fermention negative	→ 57	
57(56).	a	Vitamin-free growth positive	*C. cantarellii*:	p. 492
	b	Vitamin-free growth negative	*C. boidinii*:	p. 488
58(55).	a	Vitamin-free growth positive	*C. cantarellii*:	p. 492
	b	Vitamin-free growth negative	*C. pini*:	p. 540
59(38).	a	DL-Lactate growth positive	→ 60	
	b	DL-Lactate growth negative	→ 62	
60(59).	a	Xylitol growth positive	→ 61	
	b	Xylitol growth negative	*C. lipolytica*:	p. 520
61(60).	a	Melezitose growth positive	*C. tenuis*:	p. 561
	b	Melezitose growth negative	*C. nemodendra*:	p. 531
62(59).	a	Cellobiose growth positive	→ 61	
	b	Cellobiose growth negative	→ 63	
63(62).	a	Trehalose growth positive	*C. nemodendra*:	p. 531
	b	Trehalose growth negative	*C. fermenticarens*:	p. 504
64(30).	a	Vitamin-free growth positive	→ 65	
	b	Vitamin-free growth negative	→ 67	
65(64).	a	Soluble starch growth positive	→ 66	
	b	Soluble starch growth negative	*C. rhagii*:	p. 545
66(65).	a	Growth at 37°C positive	*C. silvicultrix*:	p. 555
	b	Growth at 37°C negative	*C. fennica*:	p. 503
67(64).	a	Cellobiose growth positive	→ 68	
	b	Cellobiose growth negative	→ 85	
68(67).	a	Galactose growth positive	→ 69	
	b	Galactose growth negative	*C. mesenterica*:	p. 525
69(68).	a	Melezitose growth positive	→ 70	
	b	Melezitose growth negative	→ 83	
70(69).	a	Raffinose growth positive	→ 71	
	b	Raffinose growth negative	→ 77	
71(70).	a	Lactose growth positive	→ 72	
	b	Lactose growth negative	→ 73	
72(71).	a	Growth at 37°C positive	*C. insectorum*:	p. 515
	b	Growth at 37°C negative	*C. tenuis*:	p. 561
73(71).	a	L-Rhamnose growth positive	→ 74	
	b	L-Rhamnose growth negative	→ 76	
74(73).	a	DL-Lactate growth positive	*C. membranifaciens*:	p. 525
	b	DL-Lactate growth negative	→ 75	
75(74).	a	*N*-Acetyl-D-glucosamine growth positive	*C. insectorum*:	p. 515
	b	*N*-Acetyl-D-glucosamine growth negative	*C. silvanorum*:	p. 554
76(73).	a	Sucrose fermentation positive	*C. membranifaciens*:	p. 525
	b	Sucrose fermentation negative	*C. friedrichii*:	p. 506
77(70).	a	L-Rhamnose growth positive	→ 78	
	b	L-Rhamnose growth negative	→ 80	
78(77).	a	Growth at 37°C positive	*C. naeodendra*:	p. 529
	b	Growth at 37°C negative	→ 79	
79(78).	a	Lactose growth positive	*C. tenuis*:	p. 561
	b	Lactose growth negative	*C. atlantica*:	p. 483
80(77).	a	Glucose fermentation positive	→ 81	
	b	Glucose fermentation negative	*C. aaseri*:	p. 476
81(80).	a	Growth at 37°C positive	→ 82	
	b	Growth at 37°C negative	*C. atmosphaerica*:	p. 483
82(81).	a	Trehalose fermentation positive	*C. diddensiae*:	p. 498
	b	Trehalose fermentation negative	*C. butyri*:	p. 491
83(69).	a	Soluble starch growth positive	→ 84	
	b	Soluble starch growth negative	*C. conglobata*:	p. 496
84(83).	a	L-Rhamnose growth positive	*C. dendronema*:	p. 498
	b	L-Rhamnose growth negative	*C. sequanensis*:	p. 551
85(67).	a	Glucose fermentation positive	*C. sophiae-reginae*:	p. 556
	b	Glucose fermentation negative	*C. psychrophila*:	p. 542
86(29).	a	Trehalose growth positive	→ 87	
	b	Trehalose growth negative	→ 169	
87(86).	a	Galactose growth positive	→ 88	
	b	Galactose growth negative	→ 147	

88(87). a Cellobiose growth positive → 89
b Cellobiose growth negative → 130
89(88). a Glucose fermentation positive → 90
b Glucose fermentation negative → 123
90(89). a Melezitose growth positive → 91
b Melezitose growth negative → 116
91(90). a Growth at 37°C positive → 92
b Growth at 37°C negative → 98
92(91). a 0.01% Cycloheximide growth positive → 93
b 0.01% Cycloheximide growth negative → 96
93(92). a Soluble starch growth positive → 94
b Soluble starch growth negative → 95
94(93). a Galactose fermentation positive .... *C. tropicalis*: p. 563
C. viswanathii: p. 570
b Galactose fermentation negative .... *C. fluviatilis*: p. 505
95(93). a Raffinose growth positive .... *C. (Pichia) guilliermondii* var. *guilliermondii*: p. 511
b Raffinose growth negative .... *C. maltosa*: p. 522
96(92). a Raffinose growth positive .... *C. melibiosica*: p. 524
b Raffinose growth negative → 97
97(96). a Growth at 40°C positive .... *C. (Clavispora) lusitaniae*: p. 521
b Growth at 40°C negative .... *C. pulcherrima*: p. 543
C. reukaufii: p. 545
98(91). a Galactose fermentation positive → 99
b Galactose fermentation negative → 112
99(98). a L-Rhamnose growth positive → 100
b L-Rhamnose growth negative → 102
100(99). a Lactose growth positive → 101
b Lactose growth negative .... *C. pseudointermedia*: p. 541
101(100). a Pellicle formation positive .... *C. intermedia*: p. 516
b Pellicle formation negative .... *C. tenuis*: p. 561
102(99). a Soluble starch growth positive → 103
b Soluble starch growth negative → 107
103(102). a DL-Lactate growth positive → 104
b DL-Lactate growth negative → 105
104(103). a Trehalose fermentation positive .... *C. shehatae* var. *lignosa*: p. 552
b Trehalose fermentation negative .... *C. lyxosophila*: p. 521
105(103). a Sucrose fermentation positive .... *C. intermedia*: p. 516
b Sucrose fermentation negative → 106
106(105). a Vitamin-free growth positive .... *C. kruisii*: p. 518
b Vitamin-free growth negative .... *C. shehatae* var. *shehatae*: p. 552
C. shehatae var. *insectosa*: p. 552
107(102). a Raffinose growth positive → 108
b Raffinose growth negative → 109
108(107). a DL-Lactate growth positive .... *C. salmanticensis*: p. 548
b DL-Lactate growth negative .... *C. intermedia*: p. 516
109(107). a 0.01% Cycloheximide growth positive → 110
b 0.01% Cycloheximide growth negative .... *C. pulcherrima*: p. 543
C. reukaufii: p. 545
C. sake: p. 547
110(109). a Xylitol growth positive → 111
b Xylitol growth negative .... *C. natalensis*: p. 530
111(110). a Trehalose fermentation positive .... *C. railenensis*: p. 544
b Trehalose fermentation negative .... *C. oleophila*: p. 533
112(98). a L-Rhamnose growth positive .... *C. tenuis*: p. 561
b L-Rhamnose growth negative → 113
113(112). a 0.01% Cycloheximide growth positive → 114
b 0.01% Cycloheximide growth negative → 115
114(113). a Xylitol growth positive .... *C. xestobii*: p. 571
b Xylitol growth negative .... *C. paludigena*: p. 536
115(113). a Xylitol growth positive .... *C. pulcherrima*: p. 543
C. reukaufii: p. 545
b Xylitol growth negative .... *C. tanzawaensis*: p. 561
116(90). a Galactose fermentation positive → 117
b Galactose fermentation negative → 120
117(116). a Growth at 37°C positive → 118
b Growth at 37°C negative → 119

118(117).	a	Raffinose growth positive	*C. guilliermondii* var. *membranifaciens (Pichia ohmeri)*:	p. 511
	b	Raffinose growth negative	*C. tropicalis*:	p. 563
119(117).	a	Vitamin-free growth positive	*C. savonica*:	p. 550
	b	Vitamin-free growth negative	*C. buinensis*:	p. 491
120(116).	a	0.01% Cycloheximide growth positive → 121		
	b	0.01% Cycloheximide growth negative	*C. torresii*:	p. 562
121(120).	a	Raffinose growth positive	*C. xestobii*:	p. 571
	b	Raffinose growth negative → 122		
122(121).	a	Xylitol growth positive	*C. santamariae*:	p. 549
	b	Xylitol growth negative	*C. zeylanoides*:	p. 571
123(89).	a	Xylitol growth positive → 124		
	b	Xylitol growth negative → 126		
124(123).	a	L-Rhamnose growth positive	*C. tenuis*:	p. 561
	b	L-Rhamnose growth negative → 125		
125(124).	a	D-Gluconate growth positive	*C. saitoana*:	p. 546
	b	D-Gluconate growth negative	*C. glaebosa*:	p. 509
126(123).	a	Melezitose growth positive → 127		
	b	Melezitose growth negative → 128		
127(126).	a	Lactose growth positive	*C. paludigena*:	p. 536
	b	Lactose growth negative	*C. castrensis*:	p. 493
128(126).	a	0.01% Cycloheximide growth positive → 129		
	b	0.01% Cycloheximide growth negative	*C. insectalens*:	p. 514
129(128).	a	DL-Lactate growth positive	*C. tepae*:	p. 562
	b	DL-Lactate growth negative	*C. zeylanoides*:	p. 571
130(88).	a	Raffinose growth positive → 131		
	b	Raffinose growth negative → 135		
131(130).	a	Growth at 37°C positive → 132		
	b	Growth at 37°C negative → 134		
132(131).	a	Glucose fermentation positive → 133		
	b	Glucose fermentation negative	*C. palmioleophila*:	p. 535
133(132).	a	Melezitose growth positive	*C. haemulonii*:	p. 511
	b	Melezitose growth negative	*C. mogii*:	p. 527
134(131).	a	Galactose fermentation positive	*C. milleri*:	p. 526
	b	Galactose fermentation negative	*C. xestobii*:	p. 571
135(130).	a	Growth at 37°C positive → 136		
	b	Growth at 37°C negative → 140		
136(135).	a	DL-Lactate growth positive → 137		
	b	DL-Lactate growth negative → 139		
137(136).	a	Growth at 40°C positive → 138		
	b	Growth at 40°C negative	*C. catenulata*:	p. 494
138(137).	a	Glucose fermentation positive	*C. albicans*:	p. 476
	b	Glucose fermentation negative	*C. caseinolytica*:	p. 492
139(136).	a	Melezitose growth positive	*C. parapsilosis*:	p. 536
	b	Melezitose growth negative	*C. zeylanoides*:	p. 571
140(135).	a	Melezitose growth positive → 141		
	b	Melezitose growth negative → 145		
141(140).	a	Glucose fermentation positive → 142		
	b	Glucose fermentation negative	*C. azyma*:	p. 485
142(141).	a	DL-Lactate growth positive → 143		
	b	DL-Lactate growth negative → 144		
143(142).	a	Growth at 35°C positive	*C. quercitrusa*:	p. 543
	b	Growth at 35°C negative	*C. sake*:	p. 547
144(142).	a	Galactose fermentation positive	*C. sake*:	p. 547
	b	Galactose fermentation negative	*C. multigemmis*:	p. 528
145(140).	a	Galactose fermentation positive	*C. humilis*:	p. 513
	b	Galactose fermentation negative → 146		
146(145).	a	0.01% Cycloheximide growth positive	*C. zeylanoides*:	p. 571
	b	0.01% Cycloheximide growth negative	*C. austromarina*:	p. 484
147(87).	a	Melezitose growth positive → 148		
	b	Melezitose growth negative → 157		
148(147).	a	L-Rhamnose growth positive → 149		
	b	L-Rhamnose growth negative → 152		
149(148).	a	Growth at 37°C positive → 150		
	b	Growth at 37°C negative → 151		
150(149).	a	Raffinose growth positive	*C. odintsovae*:	p. 532
	b	Raffinose growth negative	*C. freyschussii*:	p. 506

151(149). a DL-Lactate growth positive .... *C. maritima*: p. 523
b DL-Lactate growth negative .... *C. oregonensis*: p. 534
152(148). a Cellobiose growth positive → 153
b Cellobiose growth negative → 156
153(152). a Soluble starch growth positive .... *C. insectamans*: p. 515
b Soluble starch growth negative → 154
154(153). a DL-Lactate growth positive → 155
b DL-Lactate growth negative .... *C. reukaufii*: p. 545
155(154). a Growth at 35°C positive .... *C. quercuum*: p. 543
b Growth at 35°C negative .... *C. solani*: p. 555
156(152). a Raffinose growth positive .... *C. tsuchiyae*: p. 564
b Raffinose growth negative .... *C. musae*: p. 528
157(147). a 0.01% Cycloheximide growth positive → 158
b 0.01% Cycloheximide growth negative → 164
158(157). a L-Rhamnose growth positive .... *C. anatomiae*: p. 479
b L-Rhamnose growth negative → 159
159(158). a Xylitol growth positive → 160
b Xylitol growth negative → 161
160(159). a Trehalose fermentation positive .... *C. santamariae*: p. 549
b Trehalose fermentation negative .... *C. beechii*: p. 485
161(159). a DL-Lactate growth positive → 162
b DL-Lactate growth negative → 163
162(161). a Citrate growth positive .... *C. ancudensis*: p. 480
b Citrate growth negative .... *C. petrohuensis*: p. 539
163(159). a *N*-Acetyl-D-glucosamine growth positive .... *C. zeylanoides*: p. 571
b *N*-Acetyl-D-glucosamine growth negative .... *C. drimydis*: p. 500
164(157). a Growth at 37°C positive → 165
b Growth at 37°C negative → 168
165(164). a Glucose fermentation positive → 166
b Glucose fermentation negative .... *C. silvatica*: p. 554
166(165). a Raffinose growth positive .... *C. bombi*: p. 489
b Raffinose growth negative → 167
167(166). a Growth at 40°C positive .... *C. glabrata*: p. 508
b Growth at 40°C negative .... *C. castellii*: p. 493
168(164). a Xylitol growth positive .... *C. fructus*: p. 507
b Xylitol growth negative .... *C. suecica*: p. 560
169(86). a Galactose growth positive → 170
b Galactose growth negative → 184
170(169). a Glucose fermentation positive → 171
b Glucose fermentation negative → 178
171(170). a Galactose fermentation positive .... *C. spandovensis*: p. 558
b Galactose fermentation negative → 172
172(171). a DL-lactate growth positive .... *C. cylindracea*: p. 497
b DL-lactate growth negative → 173
173(173). a Cellobiose growth positive → 174
b Cellobiose growth negative → 175
174(173). a Raffinose growth positive .... *C. gropengiesseri*: p. 510
b Raffinose growth negative .... *C. geochares*: p. 508
175(173). a 0.01% Cycloheximide growth positive → 176
b 0.01% Cycloheximide growth negative → 177
176(175). a Xylitol growth positive .... *C. gropengiesseri*: p. 510
b Xylitol growth negative .... *C. bombicola*: p. 490
177(175). a Citrate growth positive .... *C. gropengiesseri*: p. 510
b Citrate growth negative .... *C. floricola*: p. 505
178(170). a 0.01% Cycloheximide growth positive → 179
b 0.01% Cycloheximide growth negative → 181
179(178). a Cellobiose growth positive .... *C. antillancae*: p. 481
b Cellobiose growth negative → 180
180(179). a DL-Lactate growth positive .... *C. vinaria*: p. 568
b DL-Lactate growth negative .... *C. sorbophila*: p. 557
181(178). a Growth at 37°C positive → 182
b Growth at 37°C negative → 183
182(181). a *N*-Acetyl-D-glucosamine growth positive .... *C. rugosa*: p. 546
b *N*-Acetyl-D-glucosamine growth negative .... *C. pararugosa*: p. 538
183(181). a Raffinose growth positive .... *C. apis*: p. 482
b Raffinose growth negative .... *C. galacta*: p. 507

184(169). a Raffinose growth positive → 185
b Raffinose growth negative → 186
185(184). a D-Mannitol growth positive .... *C. apicola*: p. 481
b D-Mannitol growth negative .... *C. stellata*: p. 559
186(184). a L-Rhamnose growth positive → 187
b L-Rhamnose growth negative → 188
187(186). a Cellobiose growth positive .... *C. montana*: p. 527
b Cellobiose growth negative .... *C. maris*: p. 523
188(186). a D-Mannitol growth positive → 189
b D-Mannitol growth negative → 194
189(188). a 0.01% Cycloheximide growth positive → 190
b 0.01% Cycloheximide growth negative → 191
190(189). a Glucose fermentation positive .... *C. sonorensis*: p. 556
b Glucose fermentation negative .... *C. krissii*: p. 517
191(189). a Growth at 35°C positive → 192
b Growth at 35°C negative .... *C. vini*: p. 569
192(191). a Citrate growth positive .... *C. diversa*: p. 499
C. silvae: p. 553
b Citrate growth negative → 193
193(192). a Ethanol growth positive .... *C. silvae*: p. 553
b Ethanol growth negative .... *C. karawaiewii*: p. 517
194(188). a Cellobiose growth positive .... *C. dendrica*: p. 497
b Cellobiose growth negative → 195
195(194). a Vitamin-free growth positive → 196
b Vitamin-free growth negative → 202
196(195). a Ethanol growth positive → 197
b Ethanol growth negative .... *C. amapae*: p. 479
197(196). a Growth at 37°C positive → 198
b Growth at 37°C negative → 201
198(197). a Citrate growth positive .... *C. krusei*: p. 519
C. valida: p. 566
b Citrate growth negative → 199
199(198). a Growth at 40°C positive .... *C. ethanolica*: p. 503
b Growth at 40°C negative → 200
200(199). a Propane 1, 2 diol growth positive .... *C. rugopelliculosa*: p. 545
b Propane 1, 2 diol growth negative .... *C. valida*: p. 566
201(199). a *N*-Acetyl-D-glucosamine growth positive .... *C. valida*: p. 566
b *N*-Acetyl-D-glucosamine growth negative .... *C. pseudolambica*: p. 542
202(195). a Growth at 40°C positive → 203
b Growth at 40°C negative → 204
203(202). a Glucose fermentation positive .... *C. glabrata*: p. 508
b Glucose fermentation negative .... *C. inconspicua*: p. 514
204(202). a *N*-Acetyl-D-glucosamine growth positive .... *C. valida*: p. 566
b *N*-Acetyl-D-glucosamine growth negative → 205
205(204). a Xylitol growth positive .... *C. sorboxylosa*: p. 558
b Xylitol growth negative .... *C. castellii*: p. 493

Separation of *Candida* species by physiological groups

Candida is divided into physiological groups solely for convenience and ease in identification. There is no known basis of genetic relatedness for species within each of these groups. Some species appear in more than one group because of their physiological variability. There are four species not included in the Physiological Group Keys because of their particular growth characteristics. *C. austromarina* and *C. psychrophila* do not grow at 25°C, *C. famata* var. *famata* and *C. famata* var. *flareri* have extensive physiological variability and *C. glucosophila* is osmophilic and does not grow in the usual media without additional sugar.

Key to the physiological groups:

1. a Inositol assimilated .... Group I
b Inositol not assimilated → 2
2(1). a Nitrate assimilated .... Group II
b Nitrate not assimilated → 3
3(2). a Erythritol assimilated → 4
b Erythritol not assimilated → 6
4(3). a Raffinose assimilated .... Group III
b Raffinose not assimilated → 5
5(4). a Maltose assimilated .... Group IV
b Maltose not assimilated .... Group V

6(3). a Growth at 40°C .... Group VI
b Growth absent at 40°C → 7
7(6). a Raffinose assimilated → 8
b Raffinose not assimilated → 9
8(7). a Cellobiose assimilated .... Group VII
b Cellobiose not assimilated .... Group VIII
9(7). a Cellobiose assimilated → 10
b Cellobiose not assimilated → 11
10(9). a Melezitose assimilated .... Group IX
b Melezitose not assimilated .... Group X
11(9). a Trehalose assimilated .... Group XI
b Trehalose not assimilated .... Group XII

Key to species in Group I: Growth response: inositol +. See Table 50.
1. a Nitrate assimilated → 2
b Nitrate not assimilated → 5
2(1). a Erythritol assimilated → 3
b Erythritol not assimilated .... *C. valdiviana*: p. 566
3(2). a Lactose assimilated → 4
b Lactose not assimilated .... *C. incommunis*: p. 513
4(3). a Growth at 37°C .... *C. edax*: p. 500
b Growth absent at 37°C .... *C. bertae*: p. 486
5(1). a Erythritol assimilated → 6
b Erythritol not assimilated → 9
6(5). a Growth at 37°C → 7
b Growth absent at 37°C .... *C. santjacobensis*: p. 550
7(6). a Lactose assimilated → 8
b Lactose not assimilated .... *C. chiropterorum*: p. 495
8(7). a Rhamnose assimilated .... *C. blankii*: p. 487
b Rhamnose not assimilated .... *C. auringiensis*: p. 484
9(5). a Lactose assimilated .... *C. paludigena*: p. 536
b Lactose not assimilated .... *C. castrensis*: p. 493

Table 50
Key characters of Group I *Candida* species with the following primary growth response: **inositol +**

Species	Assimilation					Growth at 37°C
	Lactose	Raffinose	L-Rhamnose	Erythritol	Nitrate	
Candida auringiensis	+	–	–	+	–	+
C. bertae	+	+	+	+	+	–
C. blankii	+	v	+	+	–	+
C. castrensis	–	–	–	–	–	–
C. chiropterorum	–	+	+	+	–	+
C. edax	+	+	+	+	+	+
C. incommunis	–	–	–	+	+	v
C. paludigena	+	v	–	–	–	–
C. santajacobensis	–	+	v	+	–	–
C. valdiviana	v	+	–	–	+	–

Key to species in Group II: Growth response: inositol –, nitrate +. See Table 51.
1. a Erythritol assimilated → 2
b Erythritol not assimilated → 5
2(1). a Sucrose assimilated → 3
b Sucrose not assimilated → 4
3(2). a Lactose assimilated .... *C. chilensis*: p. 495
b Lactose not assimilated .... *C. ishiwadae*: p. 516
4(2). a Galactose assimilated .... *C. nanaspora*: p. 530
b Galactose not assimilated .... *C. boidinii*: p. 488
5(1). a Melezitose assimilated → 6
b Melezitose not assimilated → 8
6(5). a Growth at 37°C .... *C. utilis*: p. 565
b Growth absent at 37°C → 7

7(6). a Growth present with 0.01% cycloheximide .... *C. populi*: p. 541
b Growth absent with 0.01% cycloheximide .... *C. vartiovaarae*: p. 567
8(5). a L-Rhamnose assimilated → 9
b L-Rhamnose not assimilated → 13
9(8). a Sucrose assimilated .... *C. ernobii*: p. 501
b Sucrose not assimilated → 10
10(9). a Galactose assimilated → 11
b Galactose not assimilated .... *C. norvegica*: p. 532
11(10). a Growth at 37°C .... *C. methanosorbosa*: p. 526
b Growth absent at 37°C → 12
12(11). a Cellobiose assimilated .... *C. wickerhamii*: p. 570
b Cellobiose not assimilated .... *C. nitratophila*: p. 531
13(8). a Growth at 37°C → 14
b Growth absent at 37°C → 15
14(13). a Galactose assimilated .... *C. magnoliae*: p. 521
C. vaccinii: p. 565
b Galactose not assimilated .... *C. berthetii*: p. 487
15(13). a Cellobiose assimilated → 16
b Cellobiose not assimilated → 17
16(15). a Galactose assimilated .... *C. versatilis*: p. 567
b Galactose not assimilated .... *C. norvegica*: p. 532
17(15). a Raffinose assimilated .... *C. lactis-condensi*: p. 519
b Raffinose not assimilated → 18
18(17). a Growth present with 0.1% cycloheximide → 19
b Growth absent with 0.1% cycloheximide .... *C. etchellsii*: p. 502
19(18). a Galactose assimilated .... *C. vanderwaltii*: p. 566
b Galactose not assimilated .... *C. pignaliae*: p. 539

Table 51
Key characters of Group II *Candida* species with the following primary growth responses: **inositol** −, **nitrate** +

Species	Assimilation								Growth at 37°C	0.01% Cyclo-heximide
	Galactose	Sucrose	Cellobiose	Lactose	Raffinose	Melezitose	L-Rhamnose	Erythritol		
Candida berthetii	−	−	+	−	−	−	−	−	+	+
C. boidinii	−	−	−	−	−	−	v	+	v	+
C. chilensis	+	+	+	+	−	+	−	+	−	+
C. ernobii	−	+	+	−	−	−	+	−	−	+
C. etchellsii	v	−	−	−	−	−	−	−	−	−
C. ishiwadae	v	+	+	−	−	+	+	+	−	+
C. lactis-condensi	−	+	−	−	+	−	−	−	−	v
C. magnoliae	+	v	v	−	v	−	−	−	+	v
C. methanosorbosa	+	−	v	−	−	−	+	−	+	+
C. nanaspora	+	−	−	−	−	−	+	+	+	+
C. nitratophila	+	−	−	−	−	−	+	−	−	+
C. norvegica	−	−	+	−	−	−	v	−	−	v
C. pignaliae	−	−	−	−	−	−	−	−	−	+
C. populi	−	+	+	−	−	+	+	−	−	+
C. utilis	−	+	+	−	+	+	−	−	+	−
C. vaccinii	+	+	+	−	+	−	−	−	+	+
C. vanderwaltii	+	v	−	−	−	−	−	−	−	+
C. vartiovaarae	−	+	+	−	−	+	−	−	−	−
C. versatilis	+	v	+	v	v	−	−	−	−	v
C. wickerhamii	+	−	+	−	−	−	+	−	−	+

Key to species in Group III: Growth responses: inositol −, nitrate −, erythritol +, raffinose +. See Table 52.
1. a Growth in vitamin-free medium → 2
b Growth absent in vitamin-free medium → 4

2(1). a Maltose fermented → 3
b Maltose not fermented .... *C. rhagii*: p. 545
3(2). a Trehalose fermented .... *C. fennica*: p. 503
b Trehalose not fermented .... *C. silvicultrix*: p. 555
4(1). a Raffinose fermented → 5
b Raffinose not fermented → 7
5(4). a Lactose assimilated .... *C. entomophila*: p. 500
b Lactose not assimilated → 6
6(5). a 2-Keto-D-gluconate assimilated .... *C. membranifaciens*: p. 525
b 2-Keto-D-gluconate not assimilated .... *C. silvanorum*: p. 554
7(4). a L-Rhamnose assimilated → 8
b L-Rhamnose not assimilated → 9
8(7). a Growth at 37°C .... *C. insectorum*: p. 515
b Growth absent at 37°C .... *C. tenuis*: p. 561
9(7). a Maltose fermented → 10
b Maltose not fermented .... *C. friedrichii*: p. 506
10(9). a DL-Lactate assimilated .... *C. shehatae* var. *lignosa*: p. 552
b DL-Lactate not assimilated .... *C. shehatae* var. *shehatae*: p. 552

Table 52
Key characters of Group III *Candida* species with the following primary growth responses: **inositol** –, **nitrate** –, **erythritol** +, **raffinose** +

Species	Fermentation			Assimilation				Growth in vitamin-free medium	Growth at 37°C
	Maltose	Trehalose	Raffinose	Lactose	L-Rhamnose	DL-Lactate	2-Keto-D-gluconate		
Candida entomophila	–	+	+	+	–	–	v	–	+
C. fennica	+	+	v	v	–	–	+	+	–
C. friedrichii	–	+	–	–	–	v	+	–	–
C. insectorum	–	+	–	v	+	–	–	–	+
C. membranifaciens	v	+	+	–	v	+	+	–	v
C. rhagii	–	+	v	–	v	v	+	+	–
C. shehatae var. shehatae	+	+	–	v	–	–	+	–	–
C. shehatae var. *lignosa*	+	+	–	v	–	+	+	–	–
C. silvanorum	+	+	+	–	+	–	–	–	+
C. silivultrix	+	–	+	–	–	+	–	+	+
C. tenuis	v	v	–	+	+	v	+	–	–

Key to species in Group IV: Growth responses: inositol –, nitrate –, erythritol +, raffinose –, maltose +. See Table 53.

1. a Growth at 37°C → 2
b Growth absent at 37°C → 7
2(1). a Lactose assimilated .... *C. aaseri*: p. 476
b Lactose not assimilated → 3
3(2). a L-Rhamnose assimilated → 4
b L-Rhamnose not assimilated → 6
4(3). a Trehalose fermented → 5
b Trehalose not fermented .... *C. peltata*: p. 538
5(4). a Maltose fermented .... *C. homilentoma*: p. 512
b Maltose not fermented .... *C. naeodendra*: p. 529
6(3). a Trehalose fermented .... *C. diddensiae*: p. 498
b Trehalose not fermented .... *C. butyri*: p. 491
7(1). a L-Rhamnose assimilated → 8
b L-Rhamnose not assimilated → 10
8(7). a Lactose assimilated .... *C. tenuis*: p. 561
b Lactose not assimilated → 9
9(8). a Trehalose fermented .... *C. dendronema*: p. 498
b Trehalose not fermented .... *C. atlantica*: p. 483
10(7). a Cellobiose assimilated → 11
b Cellobiose not assimilated .... *C. sophiae-reginae*: p. 556

11(10). a Trehalose fermented → 12
b Trehalose not fermented → 15
12(11). a Soluble starch assimilated → 13
b Soluble starch not assimilated → 14
13(12). a Growth in vitamin-free medium .... *C. fennica*: p. 503
b Growth absent in vitamin-free medium .... *C. shehatae* var. *shehatae*: p. 552
C. shehatae var. *lignosa*: p. 552
14(12). a Lactose assimilated .... *C. coipomoensis*: p. 496
b Lactose not assimilated .... *C. atmosphaerica*: p. 483
15(11). a Galactose assimilated → 16
b Galactose not assimilated .... *C. mesenterica*: p. 525
16(15). a Lactose assimilated .... *C. ergastensis*: p. 501
b Lactose not assimilated .... *C. sequanensis*: p. 551

Table 53

Key characters of Group IV *Candida* species with the following primary growth responses: **inositol** –, **nitrate** –, **erythritol** +, **raffinose** –, **maltose** +

Species	Fermentation		Assimilation					Growth in vitamin-free medium	Growth at 37°C
	Maltose	Trehalose	Galactose	Cellobiose	Lactose	Soluble starch	L-Rhamnose		
Candida aaseri	–	–	+	+	+	–	–	–	+
C. atlantica	–	–	+	–	–	–	+	–	–
C. atmosphaerica	v	+	+	+	–	–	–	–	–
C. butyri	–	–	+	+	–	–	–	–	+
C. coipomoensis	+	+	+	+	+	–	–	–	–
C. dendronema	v	+	+	+	–	+	+	–	–
C. diddensiae	v	+	+	+	–	–	–	–	+
C. ergastensis	–	–	+	+	+	–	–	–	–
C. fennica	+	+	+	+	v	+	–	+	–
C. homilentoma	+	+	+	+	–	+	+	v	+
C. mesenterica	–	–	–	+	–	–	–	–	–
C. naeodendra	–	+	+	+	–	+	+	–	+
C. peltata	–	–	+	+	–	+	+	–	+
C. sequanensis	–	–	+	+	–	+	–	–	–
C. shehatae var. shehatae	+	+	+	+	v	+	–	–	–
C. shehatae var. lignosa	+	+	+	+	v	+	–	–	–
C. sophiae-reginae	–	+	+	–	–	–	–	–	–
C. tenuis	v	v	+	+	+	v	+	–	–

Key to species in Group V: Growth responses: inositol –, nitrate –, erythritol +, raffinose –, maltose –. See Table 54.

1. a Glucose fermented → 2
b Glucose not fermented → 12
2(1). a Trehalose assimilated → 3
b Trehalose not assimilated .... *C. boidinii*: p. 488
3(2). a Cellobiose assimilated → 4
b Cellobiose not assimilated → 7
4(3). a Galactose assimilated → 5
b Galactose not assimilated .... *C. ovalis*: p. 535
5(4). a Growth present with 0.01% cycloheximide → 6
b Growth absent with 0.01% cycloheximide .... *C. conglobata*: p. 496
6(5). a Galactose fermented .... *C. succiphila*: p. 559
b Galactose not fermented .... *C. boleticola*: p. 489
7(3). a Galactose assimilated → 8
b Galactose not assimilated → 10
8(7). a Galactose fermented .... *C. schatavii*: p. 551
b Galactose not fermented → 9

9(8). a Growth in vitamin-free medium .... *C. cantarellii*: p. 492
b Growth absent in vitamin-free medium .... *C. boleticola*: p. 489
C. laureliae: p. 519
10(7). a Growth in vitamin-free medium .... *C. cantarellii*: p. 492
b Growth absent in vitamin-free medium → 11
11(10). a Ribitol assimilated .... *C. llanquihuensis*: p. 520
b Ribitol not assimilated .... *C. pini*: p. 540
12(1). a Trehalose assimilated .... *C. nemodendra*: p. 531
b Trehalose not assimilated → 13
13(12). a Galactitol assimilated .... *C. fermenticarens*: p. 504
b Galactitol not assimilated .... *C. lipolytica*: p. 520

Table 54
Key characters of Group V *Candida* species with the following primary growth responses: **inositol** –, **nitrate** –, **erythritol** +, **raffinose** –, **maltose** –

Species	Fermentation		Assimilation					Growth in vitamin-free medium	Growth with 0.01% cyclo-heximide
	Glucose	Galactose	Galactose	Trehalose	Cellobiose	Ribitol	Galactitol		
Candida boidinii	+	–	–	–	–	+	–	–	+
C. boleticola	+	–	+	+	v	+	–	–	+
C. cantarellii	+	–	v	+	–	v	–	+	+
C. conglobata	+	+	+	+	+	+	–	–	–
C. fermenticarens	–	–	+	–	–	+	+	–	+
C. laureliae	+	–	+	+	–	+	–	–	+
C. lipolytica	–	–	v	–	v	v	–	–	+
C. llanquihuensis	+	–	–	+	–	+	–	–	+
C. nemodendra	–	–	+	+	v	+	+	v	+
C. ovalis	+	–	–	+	+	+	–	–	+
C. pini	+	–	–	+	–	–	–	–	+
C. schatavii	+	+	+	+	–	+	–	+	+
C. succiphila	+	+	+	+	+	+	v	v	+

Key to species in Group VI: Growth responses: inositol –, nitrate –, erythritol –, 40°C +. See Table 55.

1. a Cellobiose assimilated → 2
b Cellobiose not assimilated → 11
2(1). a Galactose assimilated → 3
b Galactose not assimilated → 9
3(2). a Maltose assimilated → 4
b Maltose not assimilated .... *C. insectalens*: p. 514
4(3). a Raffinose assimilated → 5
b Raffinose not assimilated → 8
5(4). a Glucose fermented → 6
b Glucose not fermented .... *C. saitoana*: p. 546
6(5). a Melezitose assimilated → 7
b Melezitose not assimilated .... *C. (Pichia) guilliermondii* var. *membranifaciens*: p. 511
7(6). a L-Arabinose assimilated .... *C. (Pichia) guilliermondii* var. *guilliermondii*: p. 511
b L-Arabinose not assimilated .... *C. melibiosica*: p. 524
8(7). a Soluble starch assimilated .... *C. tropicalis*: p. 563
C. viswanathii: p. 570
b Soluble starch not assimilated .... *C. (Clavispora) lusitaniae*: p. 521
9(2). a Maltose assimilated → 10
b Maltose not assimilated .... *C. sonorensis*: p. 556
10(9). a Sucrose assimilated .... *C. freyschussii*: p. 506
b Sucrose not assimilated .... *C. insectamans*: p. 515
11(1). a Maltose assimilated → 12
b Maltose not assimilated → 14
12(11). a Glucose fermented → 13
b Glucose not fermented .... *C. palmioleophila*: p. 535
13(12). a Soluble starch assimilated .... *C. albicans*: p. 476
b Soluble starch not assimilated .... *C. parapsilosis*: p. 536

14(11). a Galactose assimilated → 15
b Galactose not assimilated → 16
15(14). a Trehalose assimilated .... *C. caseinolytica*: p. 492
b Trehalose not assimilated .... *C. rugosa*: p. 546
16(15). a D-Glucosamine assimilated → 17
b D-Glucosamine not assimilated → 18
17(16). a Glucose fermented .... *C. krusei (Issatchenkia orientalis)*: p. 519
b Glucose not fermented .... *C. inconspicua*: p. 514
18(16). a Growth in vitamin-free medium .... *C. ethanolica*: p. 503
b Growth absent in vitamin-free medium .... *C. glabrata*: p. 508

Table 55
Key characters of Group VI *Candida* species with the following primary growth responses: **inositol** –, **nitrate** –, **erythritol** –, **40°C** +

Species	Glucose fermentation	Assimilation[a]										Growth in vitamin-free medium
		Gal	Mal	Suc	Cel	Tre	Raf	Mz	St	L-Ar	DGM	
Candida albicans	+	+	+	v	–	+	–	v	+	v	v	v
C. caseinolytica	–	+	–	v	–	+	–	–	–	–	–	–
C. ethanolica	v	–	–	–	–	–	–	–	–	–	–	+
C. freyschussii	+	–	+	+	+	+	–	+	–	–	–	–
C. glabrata	+	–	–	–	–	v	–	–	–	–	–	–
C. guilliermondii var. guilliermondii	+	+	+	+	+	+	+	+	–	+	+	–
C. guilliermondii var. membranifaciens	+	+	+	+	+	+	+	–	–	–	+	–
C. inconspicua	–	–	–	–	–	–	–	–	–	–	+	–
C. insectalens	–	+	–	–	+	+	–	–	–	–	+	v
C. insectamans	+	–	+	–	+	+	–	+	+	–	–	–
C. krusei	+	–	–	–	–	–	–	–	–	–	+	+
C. lusitaniae	+	+	+	+	+	+	–	+	–	v	–	–
C. melibiosica	+	+	+	+	+	+	+	+	–	–	v	v
C. palmioleophila	–	+	+	+	–	+	+	+	+	–	+	–
C. parapsilosis	+	+	+	+	–	+	–	+	–	+	v	–
C. rugosa	–	+	–	–	–	–	–	–	–	–	–	–
C. saitoana	–	+	+	+	+	+	+	v	v	v	v	v
C. sonorensis	+	–	–	–	+	–	–	–	–	+	–	–
C. tropicalis	+	+	+	v	+	+	–	v	+	–	v	v
C. viswanathii	+	+	+	+	+	+	–	+	+	v	v	–

[a] Abbreviations: Gal, galactose; Mal, maltose; Suc, sucrose; Cel, cellobiose; Tre, trehalose; Raf, raffinose; Mz, melezitose; St, soluble starch; L-Ar, L-arabinose; DGM, D-glucosamine.

Key to species in Group VII: Growth responses: inositol –, nitrate –, erythritol –, 40°C –, raffinose +, cellobiose +. See Table 56.

1. a Trehalose assimilated → 2
b Trehalose not assimilated .... *C. gropengiesseri*: p. 510
2(1). a Galactose assimilated → 3
b Galactose not assimilated → 6
3(2). a L-Rhamnose assimilated → 4
b L-Rhamnose not assimilated → 7
4(3). a Growth at 37°C .... *C. (Pichia) guilliermondii* var. *guilliermondii*: p. 511
b Growth absent at 37°C → 5
5(4). a Lactose assimilated .... *C. tenuis*: p. 561
C. intermedia: p. 516
b Lactose not assimilated .... *C. pseudointermedia*: p. 541
6(2). a Growth at 37°C .... *C. odintsovae*: p. 532
b Growth absent at 37°C .... *C. maritima*: p. 523

7(3). a Glucose fermented → 8
b Glucose not fermented .... *C. glaebosa*: p. 509
C. saitoana: p. 546
8(7). a Growth at 37°C → 9
b Growth absent at 37°C → 10
9(8). a L-Arabinose assimilated .... *C. (Pichia) guilliermondii* var. *guilliermondii*: p. 511
b L-Arabinose not assimilated .... *C. melibiosica*: p. 524
10(8). a Glycerol assimilated → 11
b Glycerol not assimilated .... *C. salmanticensis*: p. 548
C. intermedia: p. 516
11(10). a Galactose fermented → 12
b Galactose not fermented .... *C. xestobii*: p. 571
12(11). a Sucrose fermented .... *C. intermedia*: p. 516
b Sucrose not fermented .... *C. shehatae*: p. 552

Table 56
Key characters of Group VII *Candida* species with the following primary growth responses: **inositol** –, **nitrate** –, **erythritol** –, **40°C** –, **raffinose** +, **cellobiose** +

Species	Fermentation			Assimilation						Growth at 37°C
	Glucose	Galactose	Sucrose	Galactose	Trehalose	Lactose	L-Arabinose	L-Rhamnose	Glycerol	
Candida glaebosa	–	–	–	+	+	+	–	–	+	–
C. gropengiesseri	+	–	+	+	–	–	–	–	+	v
C. guilliermondii var. guilliermondii	+	v	+	+	+	–	+	v	+	+
C. intermedia	+	+	+	+	+	+	v	v	v	–
C. maritima	+	–	v	–	+	–	v	+	+	–
C. melibiosica	+	+	v	+	+	–	–	–	+	+
C. odintsovae	+	–	+	–	+	–	+	+	+	+
C. pseudointermedia	+	+	+	+	+	–	–	+	–	–
C. saitoana	–	–	–	+	+	v	v	–	+	v
C. salmanticensis	+	+	+	+	+	v	–	–	–	–
C. shehatae var. shehatae	+	+	–	+	+	v	v	–	+	–
C. shehatae var. insectosa	+	+	–	+	+	v	–	–	+	–
C. shehatae var. lignosa	+	+	–	+	+	v	v	–	+	–
C. tenuis	v	v	v	+	+	+	v	+	+	–
C. xestobii	+	–	–	+	+	–	+	–	+	–

Key to the species in Group VIII: Growth responses: inositol –, nitrate –, erythritol –, 40°C –, raffinose +, cellobiose –. See Table 57.

1. a Glucose fermented → 2
b Glucose not fermented .... *C. apis*: p. 482
2(1). a Trehalose assimilated → 3
b Trehalose not assimilated → 8
3(2). a Galactose assimilated → 4
b Galactose not assimilated → 7
4(3). a Growth at 37°C → 5
b Growth absent at 37°C → 6
5(4). a Melezitose assimilated .... *C. haemulonii*: p. 511
b Melezitose not assimilated .... *C. mogii*: p. 527
6(4). a D-Xylose assimilated .... *C. xestobii*: p. 571
b D-Xylose not assimilated .... *C. milleri*: p. 526
7(3). a Growth at 37°C .... *C. bombi*: p. 489
b Growth absent at 37°C .... *C. tsuchiyae*: p. 564
8(2). a Galactose assimilated → 9
b Galactose not assimilated → 12
9(8). a Galactose fermented .... *C. spandovensis*: p. 558
b Galactose not fermented → 10

10(9). a Maltose assimilated .... *C. floricola*: p. 505
b Maltose not assimilated → 11
11(10). a Xylitol assimilated .... *C. gropengiesseri*: p. 510
b Xylitol not assimilated .... *C. bombicola*: p. 490
12(8). a Glycerol assimilated .... *C. apicola*: p. 481
b Glycerol not assimilated .... *C. stellata*: p. 559

Table 57

Key characters of Group VIII *Candida* species with the following primary growth responses: **inositol** –, **nitrate** –, **erythritol** –, **40°C** –, **raffinose** +, **cellobiose** –

Species	Fermentation		Assimilation							Growth at 37°C
	Glucose	Galactose	Galactose	Maltose	Trehalose	Melezitose	D-Xylose	Glycerol	Xylitol	
Candida apicola	+	–	–	–	–	–	v	+	v	–
C. apis	–	–	+	–	–	–	–	+	–	–
C. bombi	+	–	–	–	+	–	–	+	–	+
C. bombicola	+	–	+	–	–	–	–	+	–	–
C. floricola	+	–	+	+	–	–	v	+	v	–
C. gropengiesseri	+	–	+	–	–	–	v	+	+	v
C. haemulonii	+	–	+	+	+	+	v	+	v	+
C. milleri	+	+	+	–	+	–	–	v	–	–
C. mogii	+	–	+	+	+	–	+	+	+	+
C. spandovensis	+	+	+	–	–	–	+	+	+	–
C. stellata	+	–	–	–	–	–	–	–	–	–
C. tsuchyiae	+	–	–	+	+	+	–	+	+	–
C. xestobii	+	–	+	+	+	v	+	+	+	–

Key to species in Group IX: Growth responses: inositol –, nitrate –, erythritol –, 40°C –, raffinose –, cellobiose +, melezitose +. See Table 58.

1. a L-Rhamnose assimilated → 2
b L-Rhamnose not assimilated → 4
2(1). a Lactose assimilated .... *C. tenuis*: p. 561
b Lactose not assimilated → 3
3(2). a DL-Lactate assimilated .... *C. maritima*: p. 523
b DL-Lactate not assimilated .... *C. oregonensis*: p. 534
4(1). a Growth at 37°C → 5
b Growth absent at 37°C → 8
5(2). a Lactose assimilated .... *C. fluviatilis*: p. 505
b Lactose not assimilated → 6
6(5). a Soluble starch assimilated .... *C. viswanathii*: p. 570
b Soluble starch not assimilated → 7
7(6). a Trehalose fermented .... *C. maltosa*: p. 522
b Trehalose not fermented .... *C. (Metschnikowia) pulcherrima*: p. 543
C. (Metschnikowia) reukaufii: p. 545
8(4). a Galactose assimilated → 9
b Galactose not assimilated → 15
9(8). a Soluble starch assimilated → 10
b Soluble starch not assimilated → 12
10(9). a Trehalose fermented → 11
b Trehalose not fermented .... *C. lyxosophila*: p. 521
11(10). a Growth in vitamin-free medium .... *C. kruisii*: p. 518
b Growth absent in vitamin-free medium .... *C. shehatae*: p. 552
12(9). a Growth present with 0.01% cycloheximide → 13
b Growth absent with 0.01% cycloheximide → 16
13(12). a Xylitol assimilated → 14
b Xylitol not assimilated .... *C. natalensis*: p. 530
14(13). a Trehalose fermented .... *C. railenensis*: p. 544
b Trehalose not fermented .... *C. oleophila*: p. 533
15(8). a DL-Lactate assimilated .... *C. quercuum*: p. 543
C. solani: p. 555
b DL-Lactate not assimilated .... *C. (Metschnikowia) reukaufii*: p. 545

16(12). a Xylitol assimilated .... *C. sake*: p. 547
C. (Metschnikowia) pulcherrima: p. 543
C. (Metschnikowia) reukaufii: p. 545
b Xylitol not assimilated → 17
17(16). a Galactose fermented .... *C. sake*: p. 547
b Galactose not fermented .... *C. tanzawaensis*: p. 561

Table 58

Key characters of Group IX *Candida* species with the following primary growth responses: **inositol** –, **nitrate** –, **erythritol** –, **40°C** –, **raffinose** –, **cellobiose** +, **melezitose** +

Species	Fermentation[a]		Assimilation[a]						Growth		
	Gal	Tre	Gal	Lac	St	Rham	Lact	Xyl	Vitamin-free medium	0.01% Cycloheximide	37°C
Candida fluviatilis	–	+	+	+	+	–	+	+	–	+	+
C. kruisii	+	+	+	–	+	–	–	+	+	+	–
C. lyxosophila	+	–	+	–	+	–	+	+	–	v	–
C. maltosa	v	+	+	–	–	–	v	+	–	+	+
C. maritima	–	–	–	–	v	+	+	+	–	–	–
C. natalensis	+	–	+	–	–	–	+	–	–	+	–
C. oleophila	+	–	+	–	–	–	v	+	–	+	–
C. oregonensis	–	–	–	–	+	+	–	v	–	v	–
C. pulcherrima	v	–	+	–	–	–	v	+	–	–	v
C. quercuum	–	–	–	–	–	–	+	+	–	–	–
C. railenensis	+	+	+	–	–	–	–	+	–	+	–
C. reukaufii	v	–	v	–	–	–	–	+	–	–	v
C. sake	+	v	+	–	–	–	v	v	v	–	–
C. shehatae var. shehatae	+	+	+	v	+	–	–	+	–	+	–
C. shehatae var. insectosa	+	+	+	v	+	–	–	v	–	+	–
C. shehatae var. lignosa	+	+	+	v	+	–	+	+	–	+	–
C. solani	–	–	–	–	–	–	+	+	–	–	–
C. tanzawaensis	–	w	+	–	–	–	–	–	–	–	–
C. tenuis	v	v	+	+	v	+	v	+	–	v	–
C. viswanathii	+	+	+	–	+	–	–	+	–	+	+

[a] Abbreviations: Gal, galactose; Tre, trehalose; Lac, lactose; St, soluble starch; Rham, L-rhamnose; Lact, DL-lactate; Xyl, xylitol.

Key to species in Group X: Growth responses: inositol –, nitrate –, erythritol –, 40°C –, raffinose –, cellobiose +, melezitose –. See Table 59.

1. a Trehalose assimilated → 2
b Trehalose not assimilated → 13
2(1). a L-Rhamnose assimilated .... *C. anatomiae*: p. 479
b L-Rhamnose not assimilated → 3
3(2). a Maltose assimilated → 4
b Maltose not assimilated → 9
4(3). a Galactose assimilated → 5
b Galactose not assimilated → 6
5(4). a Glucose fermented .... *C. buinensis*: p. 491
b Glucose not fermented .... *C. tepae*: p. 562
6(4). a Growth present with 0.01% cycloheximide → 7
b Growth absent with 0.01% cycloheximide .... *C. suecica*: p. 560
7(6). a Ribitol assimilated → 8
b Ribitol not assimilated .... *C. ancudensis*: p. 480
8(7). a Citrate assimilated .... *C. drimydis*: p. 500
b Citrate not assimilated .... *C. petrohuensis*: p. 539
9(3). a Growth present with 0.01% cycloheximide → 10
b Growth absent with 0.01% cycloheximide .... *C. torresii*: p. 562
10(9). a D-Xylose assimilated → 11
b D-Xylose not assimilated → 12

11(10). a Galactose assimilated . . . . . *C. savonica*: p. 550
b Galactose not assimilated . . . . . *C. beechii*: p. 485
12(10). a Xylitol assimilated . . . . . *C. santamariae*: p. 549
b Xylitol not assimilated . . . . . *C. zeylanoides*: p. 571
13(1). a Glucose fermented → 14
b Glucose not fermented → 15
14(13). a Galactose assimilated . . . . . *C. geochares*: p. 508
b Galactose not assimilated . . . . . *C. dendrica*: p. 497
15(13). a Galactose assimilated . . . . . *C. antillancae*: p. 481
b Galactose not assimilated → 16
16(15). a L-Rhamnose assimilated . . . . . *C. montana*: p. 527
b L-Rhamnose not assimilated . . . . . *C. krissii*: p. 517

Table 59
Key characters of Group X *Candida* species with the following primary growth responses: **inositol** –, **nitrate –**, **erythritol** –, **40°C** –, **raffinose –**, **cellobiose** +, **melezitose** –

Species	Glucose fermentation	Assimilation[a]								Growth in 0.01% Cycloheximide
		Gal	Mal	Tre	Xy	Rham	Rib	Cit	Xyl	
Candida anatomiae	+	–	–	+	–	+	–	v	–	+
C. ancudensis	–	–	+	+	+	–	–	+	–	+
C. antillancae	–	+	+	–	+	–	–	–	–	+
C. beechii	+	–	–	+	+	–	+	+	+	+
C. buinensis	+	+	+	+	+	–	–	+	+	–
C. dendrica	+	–	–	–	–	–	–	+	–	+
C. drimyidis	–	–	+	+	+	–	+	+	–	+
C. geochares	+	+	–	–	–	–	+	+	+	–
C. krissii	–	–	–	–	–	–	–	+	–	+
C. montana	–	–	–	–	–	+	–	+	–	–
C. petrohuensis	–	–	+	+	+	–	+	–	–	+
C. santamariae	+	v	–	+	–	–	+	+	+	+
C. savonica	+	+	–	+	+	–	+	+	+	+
C. suecica	v	–	+	+	+	–	+	+	–	–
C. tepae	–	+	+	+	+	–	–	–	–	+
C. torresii	+	+	–	+	+	–	+	v	+	–
C. zeylanoides[b]	v	v	–	+	–	–	v	+	–	+

[a] Abbreviations: Gal, galactose; Mal, maltose; Tre, trehalose; Xy, D-xylose; Rham, L-rhamnose; Rib, ribitol; Cit, citrate; Xyl, xylitol.
[b] *C. zeylanoides* is variable at 35°C and 37°C; the remaining species in this group do not grow at these temperatures.

Key to species in Group XI: Growth responses: inositol –, nitrate –, erythritol –, 40°C –, raffinose –, cellobiose –, trehalose +. See Table 60.

1. a Melezitose assimilated → 2
b Melezitose not assimilated → 6
2(1). a Glucose fermented → 3
b Glucose not fermented . . . . . *C. azyma*: p. 485
3(2). a Galactose assimilated → 4
b Galactose not assimilated . . . . . *C. musae*: p. 528
4(3). a Growth at 37°C . . . . . *C. parapsilosis*: p. 536
b Growth absent at 37°C → 5
5(4). a L-Arabinose assimilated . . . . . *C. multigemmis*: p. 528
b L-Arabinose not assimilated . . . . . *C. quercitrusa*: p. 543
C. sake: p. 547
6(2). a Sucrose assimilated . . . . . *C. suecica*: p. 560
b Sucrose not assimilated → 7
7(6). a Growth with 0.01% cycloheximide . . . . . *C. catenulata*: p. 494
C. zeylanoides: p. 571
b Growth absent with 0.01% cycloheximide → 8
8(7). a Galactose assimilated . . . . . *C. humilis*: p. 513
b Galactose not assimilated → 9

9(8). a Growth at 37°C → 10
b Growth absent at 37°C .... *C. fructus*: p. 507
10(9). a Glucose fermented .... *C. castellii*: p. 493
b Glucose not fermented .... *C. silvatica*: p. 554

Table 60
Key characters of Group XI *Candida* species with the following primary growth responses: **inositol** –, **nitrate** –, **erythritol** –, **40°C** –, **raffinose** –, **cellobiose** –, **trehalose** +

Species	Glucose fermentation	Assimilation				Growth	
		Galactose	Sucrose	Melezitose	L-Arabinose	0.01% cyclo-heximide	37°C
Candida azyma	–	+	+	+	v	+	–
C. castellii	+	–	–	–	–	–	+
C. catenulata	v	+	–	–	–	+	+
C. fructus	+	–	–	–	–	–	–
C. humilis	+	+	–	–	–	–	–
C. multigemmis	+	+	+	+	+	–	–
C. musae	+	–	+	+	–	–	–
C. parapsilosis	+	+	+	+	+	v	+
C. quercitrusa[a]	+	+	+	+	–	v	–
C. sake[a]	+	+	+	+	–	–	–
C. silvatica	–	–	–	–	–	–	+
C. suecica	v	–	+	–	–	–	–
C. zeylanoides	v	v	–	–	–	+	v

[a] *C. quercitrusa* and *C. sake* are separated on growth at 35°C: *C. quercitrusa* +, *C. sake* –.

Key to species in Group XII: Growth responses: inositol –, nitrate –, erythritol –, 40°C –, raffinose –, cellobiose –, trehalose –. See Table 61.

It must be noted that growth with 0.01% cycloheximide was not determined for *C. amapae*. Therefore, this yeast is listed in the key as both positive and negative.

1. a Galactose assimilated → 2
b Galactose not assimilated → 8
2(1). a Sucrose assimilated .... *C. bombicola*: p. 490
b Sucrose not assimilated → 3
3(2). a Glucose fermented .... *C. cylindracea*: p. 497
b Glucose not fermented → 4
4(3). a DL-Lactate assimilated → 5
b DL-Lactate not assimilated → 7
5(4). a Growth at 37°C → 6
b Growth absent at 37°C .... *C. vinaria*: p. 568
6(5). a Growth on 50% glucose–yeast extract agar .... *C. rugosa*: p. 546
b Growth absent on 50% glucose–yeast extract agar .... *C. pararugosa*: p. 538
7(4). a Growth on 50% glucose–yeast extract agar .... *C. galacta*: p. 507
b Growth absent on 50% glucose–yeast extract agar .... *C. sorbophila*: p. 557
8(1). a Glucose fermented → 9
b Glucose not fermented → 17
9(8). a Growth in vitamin-free medium → 10
b Growth absent in vitamin-free medium → 12
10(9). a D-Xylose assimilated .... *C. pseudolambica*: p. 542
C. valida (Pichia membranifaciens): p. 566
b D-Xylose not assimilated → 11
11(10). a Glycerol assimilated .... *C. krusei*: p. 519
C. valida (Pichia membranifaciens): p. 566
b Glycerol not assimilated .... *C. rugopelliculosa*: p. 545
C. valida (Pichia membranifaciens): p. 566
12(9). a *N*-Acetyl-D-glucosamine assimilated .... *C. valida (Pichia membranifaciens)*: p. 566
b *N*-Acetyl-D-glucosamine not assimilated → 13
13(12). a D-Xylose assimilated → 14
b D-Xylose not assimilated → 15

14(13). a DL-Lactate assimilated . . . . . . *C. sorboxylosa*: p. 558
b DL-Lactate not assimilated . . . . . . *C. diversa*: p. 499
15(13). a Growth on 50% glucose–yeast extract agar . . . . . . *C. castellii*: p. 493
b Growth absent on 50% glucose–yeast extract agar → 16
16(15). a Growth at 35°C . . . . . . *C. diversa*: p. 499
C. karawaiewii: p. 517
C. silvae: p. 553
b Growth absent at 35°C . . . . . . *C. vini*: p. 569
17(8). a Growth with 0.01% cycloheximide → 18
b Growth absent with 0.01% cycloheximide . . . . . . *C. amapae*: p. 479
C. valida (Pichia membranifaciens): p. 566
18(17). a D-Xylose assimilated . . . . . . *C. maris*: p. 523
C. amapae: p. 479
b D-Xylose not assimilated . . . . . . *C. krissii*: p. 517

Table 61
Key characters of Group XII *Candida* species with the following primary growth responses[a]: **inositol** –, **nitrate** –, **erythritol** –, **40°C** –, **raffinose** –, **cellobiose** –, **trehalose** –

Species	Glucose fermentation	Assimilation[b]					Growth[c]				
		Gal	Xy	NAG	Gly	Lact	VF	50%G	0.01%	35°C	37°C
Candida amapae	–	–	+	n	–	+	+	–	n	–	–
C. bombicola	+	+	–	–	+	–	–	+	+	v	–
C. castellii	+	–	–	–	+	–	–	+	–	+	+
C. cylindracea	+	+	–	–	+	+	–	–	+	–	–
C. diversa	+	–	v	–	v	–	–	–	–	+	v
C. galacta	–	+	–	n	+	–	–	+	–	+	–
C. karawaiewii	+	–	–	–	+	–	–	–	–	w	–
C. krissii	–	–	–	+	+	–	–	+	+	–	–
C. krusei	+	–	–	+	+	+	+	v	–	+	+
C. maris	–	–	+	n	+	–	–	–	+	+	w
C. pararugosa	–	+	+	–	+	+	–	–	–	+	+
C. pseudolambica	+	–	+	–	–	+	+	–	–	+	–
C. rugopelliculosa	+	–	–	n	–	+	+	–	–	+	+
C. rugosa	–	+	v	+	+	+	–	+	–	+	+
C. silvae	+	–	–	–	+	v	–	–	–	+	v
C. sorbophila	–	+	+	–	+	–	–	–	+	+	v
C. sorboxylosa	+	–	+	–	+	+	–	v	–	+	v
C. valida	v	–	v	+	v	v	v	v	–	v	v
C. vinaria	–	+	v	+	+	+	–	–	+	–	–
C. vini	+	–	–	–	v	v	–	–	–	–	–

[a] *C. bombicola* is the only sucrose-positive yeast in this group. *C. maris* is the only L-rhamnose-positive yeast in this group.
[b] Abbreviations: Gal, galactose; Xy, D-xylose; NAG, *N*-acetyl-D-glucosamine; Gly, glycerol; Lact, DL-lactate.
[c] Abbreviations: VF, in vitamin-free medium; 50%G, on 50% (w/w) glucose–yeast extract agar; 0.01%, with 0.01% cycloheximide.

Systematic discussion of the species

64.1. *Candida aaseri* Dietrichson ex van Uden & H.R. Buckley (1970)

Synonym:
Azymocandida aaseri (Dietrichson) Novák & Zsolt (1961)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are elongate, (2.5–3.0)×(7.0–15.0) μm, in chains and clusters. A thin powdery pellicle may be present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, primitive pseudohyphae are present and consist of branched chains of elongate, sometimes rather curved cells. Aerobic growth is cream-colored, dull, soft and mostly smooth.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+/l	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	l	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	n
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Shin et al. 1996).

Mol% G+C: 37.1, CBS 1913 (T_m: Meyer and Phaff 1972).

Origin of the strain studied: CBS 1913, sputum, Norway.

Type strain: CBS 1913.

Comments: Kurtzman and Robnett (1997) found *C. aaseri* and *C. butyri* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, thus indicating the two taxa to be conspecific.

64.2. *Candida albicans* (Robin) Berkhout (1923)

Synonyms:
Oidium albicans Robin (1853)
Saccharomyces albicans (Robin) Reess (1877)
Monilia albicans Plaut (1887)
Dermatium albicans (Robin) Laurent (1889)
Monilia albicans (Robin) Zopf (1890)
Endomyces albicans (Robin) Vuillemin (1898)
Parasaccharomyces albicans (Robin) Froilano de Mello (Froilano de Mello and Gonzaga Fernandes 1918)
Monilia albicans (Robin) Zopf var. *non-liquefaciens* Sasakawa (1922)
Myceloblastanon albicans Ota (1928)
Endomyces albicans Okabe (1929)
Endomycopsis albicans (Vuillemin) Dekker (Stelling-Dekker 1931)
Mycotorula albicans (Robin) Langeron & Talice (1932)
Syringospora albicans (Robin) Dodge (1935)
Zymonema albicans (Okabe) Dodge (1935)
Mycotorula albicans (Robin) Langeron & Talice var. *vuillemini* (Landrieu ex Castellani & Chalmers) Redaelli, Ciferri & Cavallero (1938)
Procandida albicans (Robin) Novák & Zsolt (1961)
Syringospora robinii Quinquaud (1868)
Monilia candida Plaut (1885) [non Bonorden 1851]
Saccharomyces buccalis Guidi (1896)
Monilia buccalis Niño & Puglisi (1927)
Zymonema buccalis (Niño & Puglisi) Dodge (1935)
Saccharomyces tumefaciens-albus Foulerton (1900)
Myceloblastanon tumefaciens-album Ota (1928)
Cryptococcus harteri Gedoelst (1911)
Parasaccharomyces harteri Verdun (1913)
Monilia harteri (Verdun) Castellani & Chalmers (1913)
Torulopsis harteri (Verdun) Redaelli (1931)
Zymonema harteri (Verdun) Dodge (1935)
Endomyces pinoyi Castellani (1912a)
Monilia pinoyi (Castellani) Castellani & Chalmers (1913)
Blastodendrion pinoyi (Castellani) Langeron & Talice (1932)
Myceloblastanon pinoyi (Castellani) Ota (1928)
Candida pinoyi (Castellani) Basgal (1931)
Mycotorula pinoyi (Castellani) Saggese (1934)
Endomyces faecalis Castellani (1912b)
Parendomyces albus Queyrat & Laroche (1909)
Monilia alba (Queyrat & Laroche) Brumpt (1913)
Monilia decolorans Castellani & Low (1913)
Myceloblastanon decolorans (Castellani & Low) Ota (1928)
Castellania decolorans (Castellani & Low) Dodge (1935)
Endomyces vuillemini Landrieu (1912)
Guilliermondella vuillemini (Lindau) Dodge (1935)
Endomyces pulmonalis Castellani (1913)
Endomyces pulmonalis Senéz (1918) [non Castellani 1913]
Monilia pulmonalis (Castellani) Castellani & Chalmers (1913)
Candida pulmonalis (Castellani) Basgal (1931)
Castellania pulmonalis (Castellani) Dodge (1935)
Saccharomyces unguium Bourgeois (1915)
Oidiomyces unguium Frei (1921)
Onychomyces unguium Ota (1924a) nom. nud.
Monilia metchnikoffi Castellani (1916)
Castellania metchnikoffi (Castellani) Dodge (1935)
Monilia balcanica Castellani (1916)
Monilia psilosis Ashford (1917)
Myceloblastanon psilose (Ashford) Ota (1928)
Candida psilosis (Ashford) de Almeida (1933)
Syringospora psilosis (Ashford) Dodge (1935)
Parasaccharomyces ashfordi Anderson (1917)
Myceloblastanon ashfordi (Anderson) Ota (1928)
Monilia ashfordi (Anderson) Langeron & Talice (1932)
Monilia alba Castellani & Chalmers (1919)
Castellania alba (Castellani & Chalmers) Dodge (1935)

Monilia metalondinensis Castellani & Chalmers var. *alba* (Castellani & Chalmers) Castellani (1937a)
Monilia metalondinensis Castellani & Chalmers (1919)
Candida metalondinensis (Castellani & Chalmers) Berkhout (1923)
Myceloblastanon metalondinense (Castellani & Chalmers) Ota (1928)
Castellania metalondinensis (Castellani & Chalmers) Dodge (1935)
Candida albicans (Robin) Berkhout var. *metalondinensis* (Castellani & Chalmers) Ciferri (1960)
Monilia bethaliensis Pijper ex Castellani & Chalmers (1919)
Myceloblastanon bethaliensis Ota (1928)
Candida bethaliensis (Pijper) Dodge (1935)
Endomyces tropicalis Acton (1919) [non Castellani 1911]
Actonia tropicalis (Acton) Dodge (1935)
Monilia nabarroi Castellani & Chalmers (1919)
Myceloblastanon nabarroi (Castellani & Chalmers) Ota (1928)
Castellania nabarroi (Castellani & Chalmers) Dodge (1935)
Monilia pinoyi (Castellani) Castellani & Chalmers (1913) var. *nabarroi* Castellani & Chalmers) Castellani (1937a)
Monilia pseudolondinensis Castellani & Chalmers (1919)
Castellania pseudolondinensis (Castellani & Chalmers) Dodge (1935)
Monilia metalondinensis Castellani & Chalmers var. *pseudolondinensis* (Castellani & Chalmers) Castellani (1937a)
Monilia pseudolondinoides Castellani & Chalmers (1919)
Castellania pseudolondinoides (Castellani & Chalmers) Dodge (1935)
Monilia pseudometalondinensis Castellani & Chalmers (1919)
Castellania pseudometalondinensis (Castellani & Chalmers) Dodge (1935)
Endomyces actoni Vuillemin (Nannizzi 1934)
Monilia actoni (Vuillemin) Vuillemin (1931)
Monilia pseudoalbicans Neveu-Lemaire (1921)
Myceloblastanon pseudoalbicans (Neveu-Lemaire) Ota (1928)
Mycoderma pseudoalbicans (Neveu-Lemaire) Dodge (1935)
Cryptococcus copellii Froilano de Mello (Froilano de Mello and Gonzaga Fernandes 1918)
Cryptococcus copellii Neveu-Lemaire (1921)
Myceloblastanon copellii (Neveu-Lemaire) Ota (1928)
Torulopsis copellii (Neveu-Lemaire) de Almeida (1933)
Castellania copellii (Neveu-Lemaire) Dodge (1935)
Endomyces molardi Salvat & Fontoynont (1922)
Zymonema molardi (Salvat & Fontoynont) Dodge (1935)
Cryptococcus laryngitidis Sartory, Petges & Claque (1923)
Atelosaccharomyces laryngitidis (Sartory, Petges & Claque) Dodge (1935)
Monilia butantanensis Gomes (1924)
Candida butantanensis (Gomes) Langeron & Talice (1932)
Parendomyces butantanensis (Gomes) Dodge (1935)
Myceloblastanon cutaneum Ota (1924a)
Mycelorrhizodes cutaneum Ota (1924a)
Monilia cutanea (Ota) Nannizzi (1934) [non Castellani & Chalmers 1913]
Blastodendrion cutaneum (Ota) Dodge (1935)
Syringospora cutanea Dodge (1935)
Myceloblastanon gruetzii Ota (1924a)
Mycelorrhizodes gruetzii Ota (1924a)
Myceloblastanon favrei Ota (1925)
Cryptococcus favrei (Ota) Pollacci & Nannizzi (1929)
Blastodendrion favrei (Ota) Langeron & Talice (1932)
Candida favrei (Ota) de Almeida (1933)
Myceloblastanon gifuense Taniguchi (1926)
Blastodendrion gifuense (Taniguchi) Dodge (1935)
Blastodendrion intestinale Mattlet (1926)
Parasaccharomyces intestinalis (Mattlet) Dodge (1935)
Monilia richmondi Shaw (1926)
Castellania richmondi (Shaw) Dodge (1935)
Mycotorula tonsillae Carnevale-Ricci & Redaelli (Carnevale-Ricci 1926)
?*Cryptococcus tonsillarum* Nannizzi (1934)
Monilia aldoi Pereira Filho (1927)
Mycotoruloides aldoi (Pereira) Langeron & Talice (1932)
Candida aldoi (Pereira) Castellani & Jacono (1933)
Monilia fioccoi Pollacci & Nannizzi (1928)
Myceloblastanon skutetzkyi Ota (1928)
Mycocandida skutetzkyi (Ota) Dodge (1935)
Monilia mannitofermentans Castellani (1929)
Castellania mannitofermentans (Castellani) Dodge (1935)
Monilia periungealis Niño (1930)
Parendomyces periungealis (Niño) Dodge (1935)
Mycotorula periungealis Niño (1938)
Monilia alvarezsotoi Mazza & Niño (Mazza, Niño, Quintana and Bernasconi 1931)
Zymonema alvarezsotoi (Mazza & Niño) Dodge (1935)
Mycotorula alvarezsotoi (Mazza & Niño) Niño (1938)
Monilia vaginalis Mazza & de los Rios (1931)
Blastodendrion erectum Langeron & Talice (1932)
Mycotoruloides ovalis Langeron & Talice (1932)
Mycotoruloides triadis Langeron & Talice (1932)
Monilia triadis (Langeron & Talice) Castellani (1937a)
Candida triadis (Langeron & Talice) Langeron & Guerra (1938)
Monilia inexorabilis Mazza & Palamedi (1932)
Syringospora inexorabilis (Mazza & Palamedi) Dodge (1935)
Blastodendrion oosporoides Zach (Wolfram and Zach 1934a)
Parasaccharomyces oosporoides (Zach) Dodge (1935)
Cryptococcus pinoysimilis Castellani (1933)
Blastodendrion pinoysimilis (Castellani) Castellani & Jacono (1933)
Candida pinoysimilis (Castellani) Castellani & Jacono (1933)
Monilia pinoysimilis (Castellani) Castellani & Jacono (1933)
Mycocandida pinoysimilis (Castellani) Redaelli & Ciferri (Ciferri and Redaelli 1935)
Candida desidiosa Ciferri & Redaelli (1935)
Mycoderma desidiosum (Ciferri & Redaelli) Dodge & Moore (1936)
Candida mycotoruloidea Redaelli & Ciferri (Ciferri and Redaelli 1935)
Mycotorula sinensis Reiss (1935)
Mycotorula verticillata Redaelli & Ciferri (Ciferri and Redaelli 1935)
Parasaccharomyces colardi Dodge (1935)
Syringospora hasegawae Dodge (1935)
Syringospora negroni Dodge (1935)
Zymonema album Dodge (1935)
Monilia stellatoidea Jones & Martin (1938)
Candida stellatoidea (Jones & Martin) Langeron & Guerra (1939)
Candida albicans (Robin) Berkhout var. *stellatoidea* (Jones & Martin) Diddens & Lodder (1942)
Procandida stellatoidea (Jones & Martin) Novák & Zsolt (1961)
Syringospora stellatoidea van der Walt (1970g)
Candida truncata Vanbreuseghem (1948)
Candida claussenii Lodder & Kreger-van Rij (1952)
Syringospora claussenii van der Walt (1970a)
Candida intestinalis Batista & Silveira (1959c)
Candida biliaria Batista & Silveira (1959c)
Procandida langeroni (Dietrichson) Novák & Zsolt (1961)
Candida langeroni Dietrichson ex van Uden & H.R. Buckley (1970)
Candida genitalis Batista & Silveira (1962)
Procandida grubyii Novák & Vitéz (1964)
Candida nouvelii Saëz (1973a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (3.5–6.0)×(4.0–8.0) μm, single, in pairs, chains and clusters (Fig. 211).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, well-differentiated, branched pseudohyphae with chains or clusters of blastoconidia and septate hyphae are

present. Pseudohyphae of the "Mycotorula" type are often observed – grape-like clusters of blastoconidia occurring along the pseudohyphal chains. Chlamydospores are usually present. A few strains fail to form pseudohyphae when first isolated, however, most of them demonstrate this ability after being maintained in culture. Aerobic growth is white to cream-colored, glistening, usually butyrous, soft, and smooth; sometimes growth is membraneous.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	–
Sucrose	–/s	Trehalose	v
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	v	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	+/l	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+/l
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	–/l
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	–/l	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–/l

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	v
D-Glucuronate	–	50% Glucose	v
Xylitol	+	10% NaCl/5% glucose	v
L-Arabinitol	–/l	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	v	Biotin-free	–/l
Butane 2,3 diol	v	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 34.9, CSAV 29-31-1; 35.1, CBS 2712; 35.7, CBS 1905; 36.9, CBS 1899 (T_m: Stenderup and Bak 1968); 34.4–35.4, 7 strains (Nakase and Komagata 1971f); 35.9–37.3, 4 strains (T_m: Meyer and Phaff 1972); 34.3–35.6; 15 strains (T_m: Meyer et al. 1984); 32.6–34.2, 34 strains (T_m: Kamiyama et al. 1989); 35.6, 35.9, 2 strains (T_m: Su and Meyer 1991).

Origin of the strains studied: CBS 562, skin infection (Mackinnon 1936); CBS 1894, soil, New Zealand; CBS 1899, skin, received as *C. truncata* (Vanbreuseghem 1948); CBS 1905, type of *Monilia stellatoidea*; CBS 1912, from sputum, type of *C. langeroni*; CBS 1918, probably human, N.F. Conant; CBS 1949, type of *C. claussenii* and *Syringospora claussenii*; CBS 2316, CBS 2689, interdigital mycosis, type of *Myceloblastanon gruetzii*; CBS 2691, pharyngitis, received as *Saccharomyces tumefaciens-albus*; CBS 2693, nail; CBS 2694, unknown; CBS 2695, sprue, authentic strain of *Monilia psilosis*; CBS 2697, sputum, type of *Mycotoruloides triadis*; CBS 2703, tonsillo-pharyngitis, type of *C. mycotoruloides*; CBS 2707, blastomycosis, type of *Monilia alvarezsotoi*; CBS 2711, sputum, type of *Monilia richmondii*; CBS 2721, unknown; CBS 2738, child's mouth; CBS 2740, from leaf; CBS 2770, unknown; CBS 2990, sputum; CBS 5137, sputum, type of *Syringospora stellatoidea*; CBS 5982, serotype A; CBS 5983, serotype B; CBS 5990, vagina; CBS 6073, sputum; CBS 6431, bronchomycosis; CBS 6552, pharynx of *Cephalopus dorsalis*, type of *C. nouvelii*; CBS 6589, vagina; CBS 6910, urine.

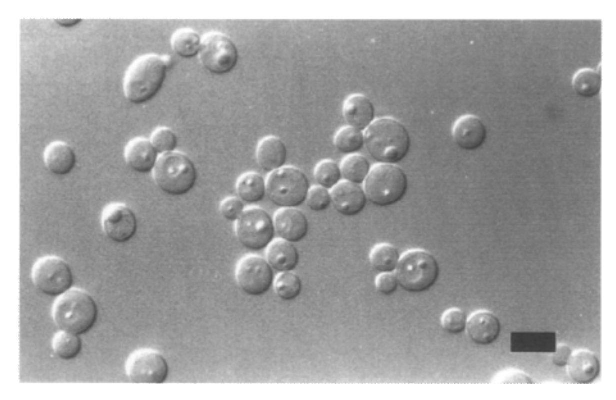


Fig. 211. *C. albicans*, CBS 562. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Type strain: CBS 562, neotype, designated by Diddens and Lodder (1942).

Comments: *C. albicans* is the most studied of all *Candida* species. The essence of much of the research is to ascertain the opportunistic or pathogenic aspects of this species, the ways to deal with the microbe/patient relationship and the epidemiology. A significant number of *C. albicans* strains are isolated routinely from clinical sources. The degree of variability has been reported repeatedly using various techniques. The terms 'typical' and 'atypical' frequently appear in publications. Besides the usual morphological and physiological characterizations as described above, *C. albicans* has been studied with numerous molecular biological techniques to determine strain identity and strain variability: restriction fragment length polymorphisms (RFLPs), karyotypes, DNA probes, randomly amplified polymorphic DNA (RAPD) analysis, nuclear DNA reassociation, mitochondrial DNA RFLPs, size and mol% G+C, protein electrophoretic profiles, as well as the phylogenetically determining criterion, ribosomal RNA gene sequences.

The conspecificity of *C. albicans* and *C. stellatoidea* was established by DNA reassociation (Meyer 1979), supported by other studies (immunoelectrophoretic analysis, Montrocher 1980) and confirmed by additional

DNA reassociation studies (Kamiyama et al. 1989). Nevertheless, Kwon-Chung et al. (1988) showed two distinct karyotype profiles for strains of *C. stellatoidea.* They were designated type I and type II. Mahrous et al. (1990) demonstrated that phenotypically diverse strains of *C. albicans,* whose identification was verified by DNA reassociation, had varying karyotypes. Sullivan et al. (1995) showed that the nucleotide sequences of the V3 region of the ribosomal RNA genes of *C. stellatoidea* type I and type II are identical and differed from five other strains of *C. albicans* at one or two nucleotide positions.

Sullivan et al. (1995) described *C. dubliniensis,* a new species isolated from sixty HIV-infected and three HIV negative persons, that closely resembles *C. albicans. C. dubliniensis* is germ tube positive and produces chlamydospores in abundance. It is serotype A and its physiological profile shows few differences compared with four reference strains (*C. albicans* serotype A and serotype B; *C. stellatoidea* type I and type II). Several molecular biological techniques (DNA fingerprinting, DNA probing, RAPD analysis, karyotyping, rRNA gene sequencing) were employed to compare strains of this species with *C. albicans* reference strains. The results showed that the strains of *C. dubliniensis* were distinct from the *C. albicans* strains. The most convincing evidence that *C. dubliniensis* is distinct from *C. albicans* is the number of nucleotide differences (13–15) in the ribosomal RNA gene sequences. However, Sullivan et al. (1995) did not compare their strains with strains representing the many synonyms of *C. albicans,* so it is possible that the species may be found to be synonymous with an earlier described species.

64.3. *Candida amapae* Morais, Rosa, S.A. Meyer, Mendonça-Hagler & Hagler (1995)

The following description is taken from the original description (Morais et al. 1995).

Growth in malt extract: After 3 days at 25°C, the cells are large and globose to elongate. After four weeks, a sediment and a thick ring are present.

Growth on malt extract agar: After 3 days, cells are pleomorphic and cylindrical to elongate. Well-developed, moderately branched pseudohyphae are also present. The streak culture is dry, white, cottony, rough with a smooth center and an irregular fringed margin. After 10 days, the growth is off-white, smooth and with a rugose lobate margin that is fringed with pseudomycelium.

Dalmau plate culture on corn meal agar: After 5 days at 25°C, abundant pseudohyphae are present. After 10–20 days, rare true hyphae are observed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	n
Inulin	–	D-Gluconate	n
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	–
Glucono-δ-lactone	–	Growth without methionine	–
Ethylacetate	–	Growth without cysteine	–
Nitrite	–	Growth without histidine	+
Cadaverine	–	Growth without DL-tryptophane	+
L-Lysine	–	Growth at 28°C	+
Ethylamine	–	Growth at 37°C	–
50% Glucose	–		
10% NaCl	–		
Starch formation	–		

Co-Q: Not determined.
Mol% G+C: 38.8, CBS 7872 (T_m: Morais et al. 1995).

Origin of the strains studied: CBS 7872 (type strain) and 13 other strains, fallen fruit of *Parahancornia amapa,* Mocambo forest, Belem, Para, Brazil.

Type strain: CBS 7872.

Comments: Morais et al. (1995) studied 14 strains of this species. Only one strain grew on succinate, citrate and glucosamine. This species is very similar to *C. sorboxylosa* and also to species of *Issatchenkia.* What differentiates *C. amapae* from these yeasts is its absolute requirement for the sulfur-containing amino acids methionine and cysteine.

64.4. *Candida anatomiae* (Zwillenberg) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis anatomiae Zwillenberg (1966)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to short-ovoidal, (3.0–5.0)×(3.0–7.0) μm, single, in pairs and short chains.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, primitive pseudohyphae of short chains of cells are sometimes present. Aerobic growth is white to cream-colored, soft, glistening, smooth and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	+/l	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	l
L-Arabinose	–	Citrate	–/l
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	+
Saccharate	–	50% Glucose	–
D-Glucoronate	–	10% NaCl/5% glucose	n
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	l	Pyridoxine-free	+
Butane 2,3 diol	l	0.01% Cycloheximide	+
Nitrite	+	0.1% Cycloheximide	–
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 39.0, CBS 5547 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 5547, corpse embalmed in formalin.

Type strain: CBS 5547.

64.5. *Candida ancudensis* C. Ramírez & A. González (1984i)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.5–4.5)×(5.0–8.0) µm; a few cells are elongate and polymorphic and single or in pairs (Fig. 212).

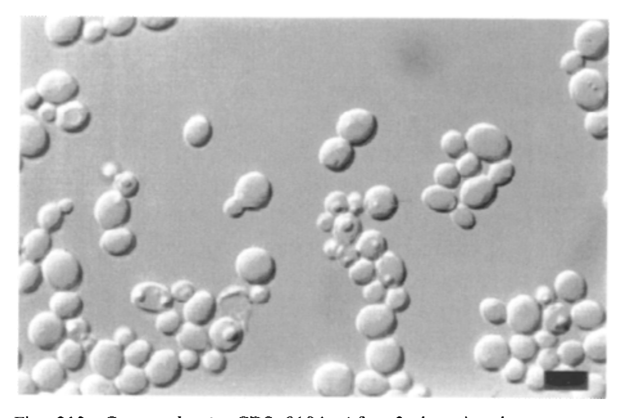


Fig. 212. *C. ancudensis*, CBS 8184. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, short primitive pseudohyphae are present. Aerobic growth is white to cream-colored, butyrous and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+/l
Melezitose	–	Salicin	l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	l
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	–
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 42.0, CBS 8184 (T_m: O'Neill and Meyer, unpublished data).

Origin of the strains studied: CBS 8184, from rotten *Drimys winteri*, Chile.

Type strain: CBS 8184.

Comments: Kurtzman and Robnett (1997) found *C. petrohuensis*, *C. ancudensis* and *C. drimydis* to have identical sequences in a 600-nucleotide region at the 5′ end of large

subunit (26S) rDNA, thus indicating the three taxa to be conspecific.

64.6. *Candida antillancae* C. Ramírez & A. González (1984a)

Synonym:
Candida bondarzewiae C. Ramírez & A. González (1984a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 20°C, the cells are ovoidal, (3.0–4.5)×(3.5–10.0) µm, single, in pairs and small groups (Fig. 213).

Dalmau plate culture on corn meal agar: After 7 days at 22°C, primitive to well-developed pseudohyphae are evident. Aerobic growth is white to cream-colored, butyrous, soft and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	l	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	l	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	l	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	l
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+/l
D-Xylose	+	Succinate	–/l
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+/w
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	s	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 25°C	w
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 39.0, CBS 8171 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 8170, CBS 8171 (type strain, *C. bondarzewiae*) rotten wood, Chile.

Type strain: CBS 8170.

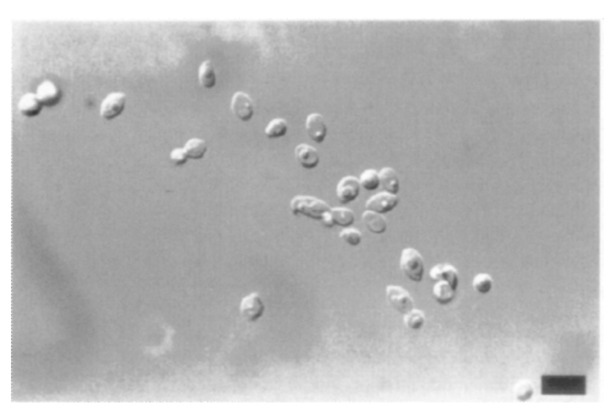


Fig. 213. *C. antillancae*, CBS 8171. After 3 days in glucose–yeast extract–peptone broth at 20°C. Bar = 5 µm.

Comments: The strains examined grow poorly at 25°C, therefore, most tests were incubated at 17–20°C. Dalmau plate cultures were incubated at 22°C. Some assimilation responses differ from those in the original descriptions of *C. antillancae* and *C. bondarzewiae*. Primarily, latent growth was evident in galactose, sucrose, cellobiose, salicin and lactic acid for *C. antillancae* and latent growth on cellobiose, salicin, arbutin and mannitol was noted for the strain of *C. bondarzewiae*, as well as positive responses on glycerol. These two yeasts are nearly identical in the tested morphological and physiological properties. In addition, Kurtzman and Robnett (1997) found *C. antillancae*, *C. bondarzewiae* and *C. tepae* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, thus indicating the three taxa to be conspecific.

64.7. *Candida apicola* (Hajsig) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:
Torulopsis apicola Hajsig (1958)
Torulopsis bacillaris (Kroemer & Krumbholz) Lodder var. *obesa* Y. Ohara, Nonomura & Yunome (1960a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spherical to ovoidal, (1.5–2.5)×(3.0–4.0) µm, and occur singly and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is yellowish to cream-colored, glistening, soft, smooth and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–/s
Sucrose	s	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	+/l	Ethanol	–/l
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–/l
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–/l	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	–/l	Starch formation	–
L-Arabinitol	–/l	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–
Nitrite	–	Growth at 30°C	+/w
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (F.-L. Lee et al. 1993).

Mol% G+C: 46.3, CBS 2868 (T_m: Stenderup et al. 1972); 44.3, CBS 2868 (HPLC: F.-L. Lee et al. 1993).

Origin of the strains studied: CBS 1887, CBS 1888, CBS 1889, pickled cucumbers, U.S.A.; CBS 2868, 2869, bee's gut; CBS 4078, grape must, type of *Torulopsis bacillaris* var. *obesa*; CBS 4353, sugar.

Type strain: CBS 2868.

64.8. *Candida apis* (Lavie ex van Uden & Vidal-Leiria) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Paratorulopsis apis (Lavie) Novák & Zsolt (1961)

Torulopsis apis Lavie ex van Uden & Vidal-Leiria (1970)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (2.0–4.0)×(3.0–7.0) μm, single, in pairs and short chains (Fig. 214).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, primitive pseudohyphae of short chains of cells are sometimes present. Aerobic growth is cream-colored to beige, soft, smooth and entire.

Fermentation: absent.

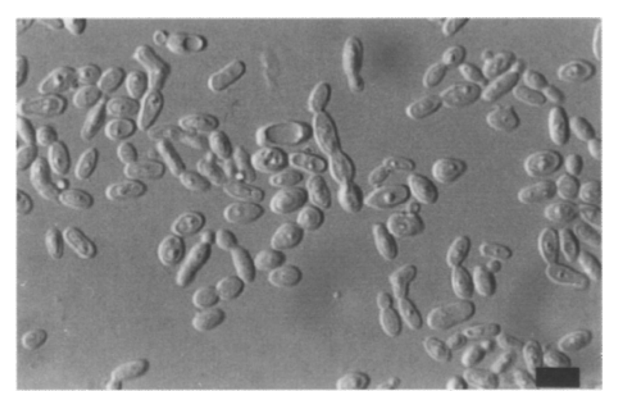


Fig. 214. *C. apis*, CBS 2674. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	l	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+/l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–
Nitrite	–	Growth at 30°C	+
Cadaverine	+	Growth at 35°C	w
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (F.-L. Lee et al. 1993).

Mol% G+C: 48.8, CBS 2674 (BD: Stenderup et al. 1972); 45.6, CBS 2674 (HPLC: F.-L. Lee et al. 1993).

Origin of the strain studied: CBS 2674, from bees.

Type strain: CBS 2674.

Comments: This species no longer has the variety *galacta*. F.-L. Lee et al. (1993) found that the mol% G+C contents of *C. apis* var. *apis* and *C. apis* var. *galacta* differed by 5.6% and Co-Q also differed, 9 and 8, respectively. DNA reassociation confirmed the lack of

relatedness between these two organisms. These investigators recognized *C. apis* var. *galacta* as the distinct species, *C. galacta*.

64.9. *Candida atlantica* (Siepmann) S.A. Meyer & Simione ex S.A. Meyer & Yarrow (1998)

Synonyms:

Trichosporon atlanticum Siepmann (Siepmann and Höhnk 1962)
Candida atlantica (Siepmann) S.A. Meyer & Simione (1978) nom. inval.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (2.5–5.0)×(2.5–6.0) µm, and single, in pairs, short chains and small clusters.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, primitive to well-developed pseudohyphae are formed. Aerobic growth is cream-colored, somewhat glistening and soft with some 'craters' in the central area of the streak. The margin is entire.

Fermentation:

Glucose	w/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–/l	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	n
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 35.8, CBS 5263 (T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 5263, shrimp eggs from the North Atlantic Ocean.

Type strain: CBS 5263.

64.10. *Candida atmosphaerica* Santa María (1959c)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to cylindrical, (1.5–3.0)×(3–12) µm, single, in pairs and chains.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, well-developed pseudohyphae of long, branched chains of cells, usually with few blastoconidia, are present. Aerobic growth is white to cream-colored, soft, smooth and butyrous.

Fermentation:

Glucose	s	Lactose	–
Galactose	–/s	Raffinose	–
Sucrose	–/s	Trehalose	s
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+/l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/l
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+/l	Inositol	–
D-Ribose	+	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–/l	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	l
Xylitol	+	10% NaCl/5% glucose	l
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 39.0, 38.4, CBS 4547 (T_m: Meyer and Phaff 1972, HPLC: C.-F. Lee et al. 1993, respectively), 39.7, CSAV 29-50-1 (T_m: Stenderup and Bak 1968); 38.8, AJ 5107 (T_m: Nakase and Komagata 1971f).

Origin of the strains studied: CBS 4547, atmosphere; CBS 7170, fruiting body of a fungus (*Tyromyces ptychogaster*) on a fallen tree trunk.

Type strain: CBS 4547.

Comments: Triangular to clover-leaf shaped cells may

be found on some media. DNA reassociation experiments confirmed that this species was not a synonym of *C. diddensiae* (Meyer and Simione 1978). C.-F. Lee et al. (1993) showed insignificant DNA relatedness between *C. atmosphaerica* and *C. dendronema* and between *C. atmosphaerica* and *C. terebra*.

64.11. *Candida auringiensis* Santa María (1978)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.5–4.0)×(3.0–5.0) μm, single and in pairs. Some cells may be teardrop-shaped.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, primitive to well-developed pseudohyphae are present. Aerobic growth is white, creamy, smooth and glistening. The margin is mostly entire with an occasional tuft of mycelial growth.

Fermentation:

Glucose	+	Lactose	s
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+/s
Maltose	–	Cellobiose	s

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	l	Inositol	+
D-Ribose	–/l	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	+
D-Glucuronate	l	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	n
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 40.2, CBS 6913; 40.0, CBS 6919 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 6913, CBS 6919, CBS 6920, from alpechin (waste from production of olive oil), Spain.

Type strain: CBS 6913.

64.12. *Candida austromarina* (Fell & I.L. Hunter) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis austromarina Fell & I.L. Hunter (1974)

Growth in glucose–yeast extract–peptone broth: After 3 days at 15°C, the cells are globose to ovoidal, (4.0–6.0)×(4.0–7.0) μm, single and in pairs. The buds may be on short stalks. A sediment is present, but no ring or pellicle.

Dalmau plate culture on corn meal agar: After 7 days at 15°C, pseudohyphae are not present. Aerobic growth is white, smooth, soft, with an entire margin.

Fermentation:

Glucose	–/s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	–	Glycerol	l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+/l	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	L-Lysine	–
5-Keto-D-gluconate	n	Ethylamine	–
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–/l
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	–	Growth at 25°C	–
Creatinine	–		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 39.0, 2 strains (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 6179, 6588, Antarctic Ocean.

Type strain: CBS 6179.

Comments: All tests reported here were conducted at 15–17°C. The maximum temperature for growth of this species is 18–21°C. Kurtzman and Robnett (1997) found *C. sake* and *C. austromarina* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.13. *Candida azyma* (van der Walt, E. Johannsen & Yarrow) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis azyma van der Walt, E. Johannsen & Yarrow (1978)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to long ovoidal, (2.0–4.0)×(5.0–9.0) μm, single, in pairs or short chains (Fig. 215).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae, if present, consist of branched chains of ovoid cells. Aerobic growth is slightly beige to cream-colored, smooth and shiny. The margin may be smooth to undulating.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	+/l
Trehalose	+	Galactitol	+/l
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–/l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–/l	Citrate	–
D-Arabinose	–/l	Inositol	–
D-Ribose	–	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

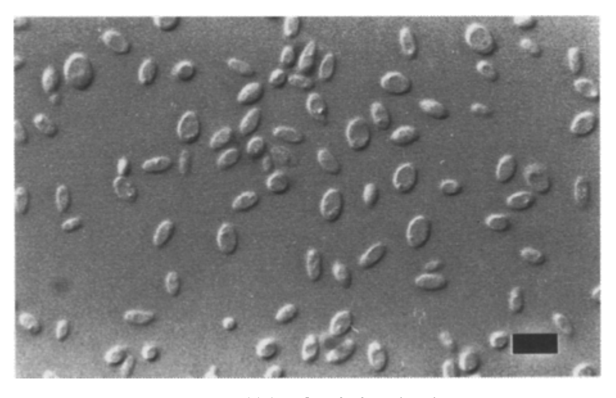


Fig. 215. *C. azyma*, CBS 6826. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	+	Starch formation	–
L-Arabinitol	l	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	–
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	–	Growth at 30°C	+/w
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 54.4, CBS 6826 (T_m: van der Walt et al. 1978).

Origin of the strains studied: CBS 6825, CBS 6826, lichen, South Africa.

Type strain: CBS 6826.

64.14. *Candida beechii* H.R. Buckley & van Uden (1968)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are cylindroidal, (2.0–3.0)×(11.0–22.0) μm, single, in pairs and chains.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, primitive to well-developed pseudohyphae are present and consist of long branched chains of cells, usually without blastoconidia. Aerobic growth is off-white to cream-colored, shiny, delicately wrinkled, and soft with a mycelial edge.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+/l	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	l	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	s
Xylitol	l	10% NaCl/5% glucose	n
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 41.2, CBS 4261 (BD: Meyer et al. 1984); 40.5, CBS 4261 (T_m: Stenderup et al. 1972).

Origin of the strain studied: CBS 4261, cider, U.K.

Type strain: CBS 4261.

Comments: Kurtzman and Robnett (1997) found *C. beechii* and *C. santamariae* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.15. *Candida bertae* C. Ramírez & A. González (1984c)

Synonym:

Candida bertae C. Ramírez & A. González var. *chiloensis* C. Ramírez & A. González (1984c)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (1.5–5.0)×(2.5–10.8) μm, and occur singly, in pairs and chains (Fig. 216). Pseudohyphae may be present. A pellicle and sediment are present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, well-developed pseudohyphae are present. True hyphae may also be present. Aerobic growth is white to cream-colored, smooth, dull and with a serrated margin.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	v	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/l
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–/l
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+/l
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	+
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

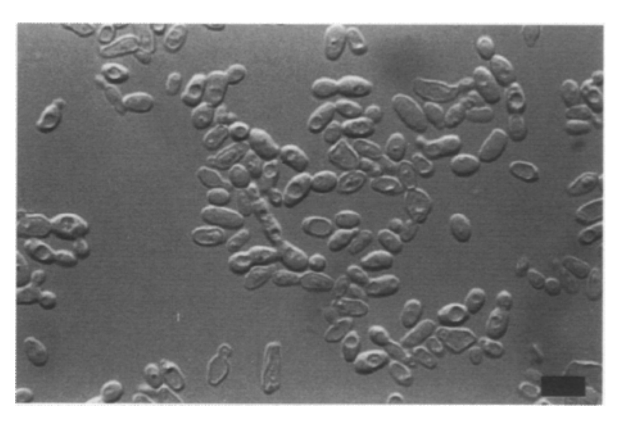


Fig. 216. *C. bertae*, CBS 8169. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	l	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–/l	Biotin-free	–
Butane 2,3 diol	v	Pyridoxine-free	+
Nitrite	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 43.7, 44.4, CBS 8169; 43.9, 43.4, CBS 8168 (T_m: M.Th. Smith, personal communication; T_m: Tengku Zainal Mulok 1988, respectively).

Origin of the strains studied: CBS 8169, CBS 8168, rotten wood in Chile.

Type strain: CBS 8169.

Comments: At the time Ramírez and González (1984c) described *C. bertae*, they also described *C. bertae* var. *chiloensis*. The variety *chiloensis* was distinguished from the variety *bertae* on the basis of trehalose assimilation and the inability to utilize glycerol, ribitol, and salicin. We found that both varieties assimilate glycerol, ribitol and salicin, either well or latently. They differ in the assimilation of maltose and trehalose. The strain designated *C. bertae* var. *chiloensis* grows well on maltose and trehalose, in contrast to the variety *bertae* which grows only latently or not at all. Other differences from the original description include the assimilation of L-sorbose, lactose, melezitose, D-ribose, and D-arabinose. Tengku Zainal Mulok (1988) found 97.6% DNA relatedness for these yeasts. We feel there are insufficient differences to warrant varietal status for the strain designated *C. bertae* var. *chiloensis*.

64.16. *Candida berthetii* Boidin, Pignal, Mermier & Arpin (1963)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (4.0–6.0)×(4.5–6.5) μm, single and in pairs. A dry pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, short densely branched pseudohyphae usually with verticils of globose blastoconidia are present. Aerobic growth is off-white to cream-colored, soft, mostly smooth and the margin entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–/l
Melibiose	–	D-Glucitol	–/l
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–/l	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	n
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+/l	Biotin-free	–
Butane 2,3 diol	–/l	Pyridoxine-free	+
Nitrite	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 39.8, type strain (T_m: Stenderup et al. 1972).

Origin of the strains studied: CBS 5452, CBS 5453, gum on tree; CBS 6112, CBS 6113, CBS 6229, insect tunnels, South Africa.

Type strain: CBS 5452.

64.17. *Candida blankii* H.R. Buckley & van Uden (1968)

Synonym:

Candida hydrocarbofumarica K. Yamada, Furukawa & Nakahara ex C. Ramírez (1974)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spheroidal to ovoidal to elongate, (2.0–5.0)×(2.0–13.0) μm, and occur singly, in pairs and short chains.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cells with blastoconidia formed in verticils. True hyphae may be present. Aerobic growth is white to cream-colored, soft, and smooth to wrinkled.

Fermentation:

Glucose	–/s	Lactose	–
Galactose	–/s	Raffinose	–
Sucrose	s	Trehalose	–
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+/l
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+/l
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–/l
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	+/l	Hexadecane	+
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	+	50% Glucose	v
Xylitol	+/l	10% NaCl/5% glucose	+/l
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 55.1, CBS 1898 (T_m: Meyer et al. 1984); 56.6, CBS 6734 (BD: Meyer et al. 1984); 56.6, AJ 5204 (T_m: Nakase and Komagata 1971f); 54.1, CBS 6427, CBS 6788; 54.4, CBS 7205 (T_m).

Origin of the strains studied: CBS 1898, mink, Canada; CBS 6734 (type strain of *C. hydrocarbofumarica*); 6427, CBS 6788, CBS 6789, soil, Japan; CBS 7205, horse, New Zealand.

Type strain: CBS 1898.

Comments: *C. hydrocarbofumarica* is reduced to synonymy with *C. blankii* because of the high degree (>90%) of nuclear DNA reassociation between the type strains in experiments conducted in the laboratories at the CBS and Georgia State University.

64.18. *Candida boidinii* C. Ramírez (1953)

Synonyms:
Candida koshuensis Yokotsuka & S. Goto (1955)
Candida olivarium Santa María (1958a)
Candida alcomigas Urakami (1975)
Candida methanolica Oki & Kounu (Oki et al. 1972)
Candida methylica Trotsenko & Bykovskaya (Trotsenko et al. 1974)
Hansenula alcolica Urakami (1975)
Torulopsis enokii Urakami (1975)
Candida queretana Herrera & Ulloa (1978)
Candida silvicola Shifrine & Phaff var. *melibiosica* Nowakowska-Waszczuk & Pietka (1983)
Candida ooitensis Kumamoto & Seriu (Kumamoto et al. 1986)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are long-ovoidal to cylindrical, (2.0–4.0)×(4.0–20.0) μm, single, in pairs and chains. A pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consisting of long branched chains of cells with verticils of ovoid blastoconidia are present. Septate hyphae may be present. Aerobic growth is off-white, cream-colored or beige, moist, shiny to dull, butyrous to membraneous, and with an irregular or a fringed margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	+
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+/l
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	–
D-Arabinose	–/l	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	v
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–/l	Pyridoxine-free	+
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	+	Growth at 35°C	v
Cadaverine	+	Growth at 37°C	v
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (J.-D. Lee and Komagata 1980a, Lin et al. 1995).

Mol% G+C: 30.5, AJ 4778, 32.4, AJ 4939 (T_m: Nakase and Komagata 1971f), 33.0, CBS 2428 (T_m: Meyer and Phaff 1972); 31.0, CBS 2428 & CBS 8051, 30.8, CBS 8030 (HPLC: C.-F. Lee et al. 1994b); 34.6, CBS 7299 (T_m: Kumamoto et al. 1986); 35.6, CBS 8030 (T_m: Trotsenko et al. 1974); 31.0–32.9, 19 strains (HPLC: Lin et al. 1995).

Origin of the strains studied: CBS 2428, tanning fluid; CBS 2429, soil; CBS 3092, olives (type strain of *C. olivarium*); CBS 5325, sea water; CBS 5777, wine (type strain of *C. koshuensis*); CBS 6056, ginger ale, U.S.A.; CBS 6202, floor of hospital ward in Finland; CBS 6295, soil; CBS 6368, washed soft-drink bottles; CBS 6990, tepache in Mexico (type strain of *C. queretana*); CBS 7299, slimy mud in Japan (type strain of *C. ooitensis*); CBS 8051 (NRRL Y-8025), 8052 (NRRL Y-8023), unknown; CBS 8106, soil; CBS 8251, soil (type strain of *C. silvicola* var. *melibiosica*); CBS 8030, soil, (type strain of *C. methylica*).

Type strain: CBS 2428.

Comments: *C. methylica* was added to the list of synonyms because of the significant DNA hybridization with the type strain of *C. boidinii* (C.-F. Lee et al. 1994b). The type strain of *C. methylica* assimilates rhamnose and not ribose whereas all other strains of *C. boidinii* assimilate ribose and not rhamnose. Kurtzman (personal communication) found the ribosomal RNA gene sequences to be identical for the type strains of *C. ooitensis* and *C. boidinii*. Their morphological and physiological properties are nearly the same with the exception of nitrate utilization. This means that the type strain of *C. ooitensis* is the only strain presently included in *C. boidinii* that is nitrate negative. Interestingly, *C. ooitensis* does assimilate nitrite. J.-D. Lee and Komagata (1983) compared the electrophoretic patterns of enzymes of *C. boidinii* strains and showed the presence of two distinct groups. Lin et al. (1996) employed several molecular and chemical techniques to examine nineteen strains of *C. boidinii* and found a high degree of genetic heterogeneity. The strains clustered into two groups according to their different electrophoretic enzyme patterns and profiles of the restriction digests of the rDNA spacer region. They also found a unique chromosomal banding pattern for each strain.

64.19. *Candida boleticola* Nakase (1971b)

Synonym:

Candida ralunensis C. Ramírez & A. González (1984f)

Growth in glucose–yeast extract–peptone broth: After 5 days at 25°C, the cells are ovoidal to elongate, (2.5–5.0)×(5.0–10.0) μm, and occur singly, in pairs and short chains (Fig. 217). The type strain forms a thin pellicle.

Dalmau plate culture on corn meal agar: After 5 days at 25°C, well-developed pseudohyphae consisting of branched chains of cylindroidal cells with small verticils of ovoidal blastoconidia are present. Aerobic growth is off-white to cream-colored, dry, wrinkled, dull and with a fringed margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+/l
Sucrose	–	Glycerol	+/l
Maltose	–	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–/l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	v
D-Glucuronate	–	10% NaCl/5% glucose	v
Xylitol	+	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	–	Growth at 30°C	v
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 43.2–44.1, 3 strains (T_m: Nakase 1971b); 44.4, CBS 6420; 43.6, CBS 8179 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 6420, from the fruiting body of the fungus *Astraeus hygrometricus*; CBS 8179, type strain of *C. ralunensis*, rotten trunk of *Laurelia sempervirens*, Chile.

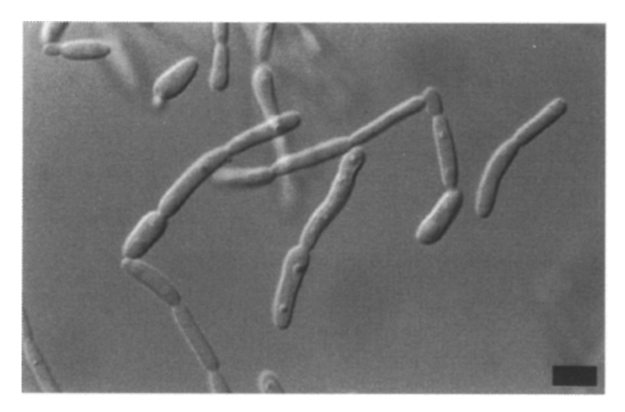


Fig. 217. *C. boleticola*, CBS 6420. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Type strain: CBS 6420.

Comments: Kurtzman and Robnett (1997) found *C. laureliae* and *C. ralunensis* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA and to differ by only one nucleotide from *C. boleticola*, thus indicating the three taxa to be conspecific. *C. boleticola* assimilates L-sorbose, D-arabinose, D-ribose, D-glucosamine, and D-gluconate, grows on high osmotic pressure media (50% glucose and 10% NaCl/5% glucose) and grows at 35°C; *C. ralunensis* does not assimilate these compounds and is unable to grow on high osmotic pressure media and at 30°C.

64.20. *Candida bombi* Montrocher (1967)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–4.0)×(4.0–6.0) μm, single, in pairs and small groups (Fig. 218).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae may be sparse or consist of branched chains of cells, sometimes with verticils of blastopores. Aerobic growth is cream-colored to light tan, smooth and soft with an entire to slightly irregular margin.

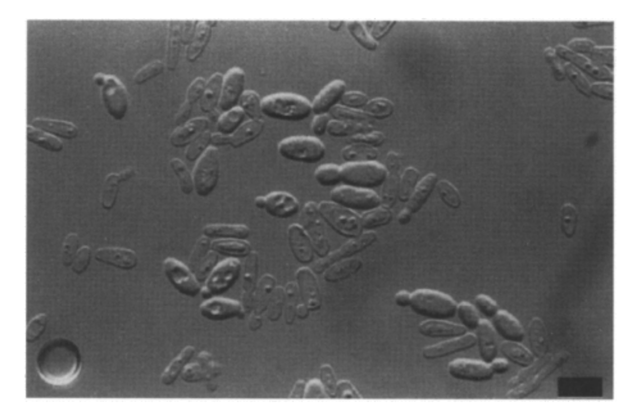


Fig. 218. *C. bombi*, CBS 5836. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	s
Sucrose	+	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	–	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (F.-L. Lee et al. 1993).

Mol% G+C: 48.3, 46.6, CBS 5836 (T_m: Meyer and Phaff 1972; HPLC: F.-L. Lee et al. 1993, respectively).

Origin of the strain studied: CBS 5836, bumble bee (*Bombus* sp.).

Type strain: CBS 5836.

64.21. *Candida bombicola* (J.F.T. Spencer, Gorin & Tulloch) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis bombicola J.F.T. Spencer, Gorin & Tulloch (1970)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to elongate, (1.5–2.5)×(3.0–5.0) μm, single and in pairs (Fig. 219).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae are absent or consist of very short densely branched chains of cells. Aerobic growth is cream-colored to gray, smooth, shiny, convex with an entire to undulating margin.

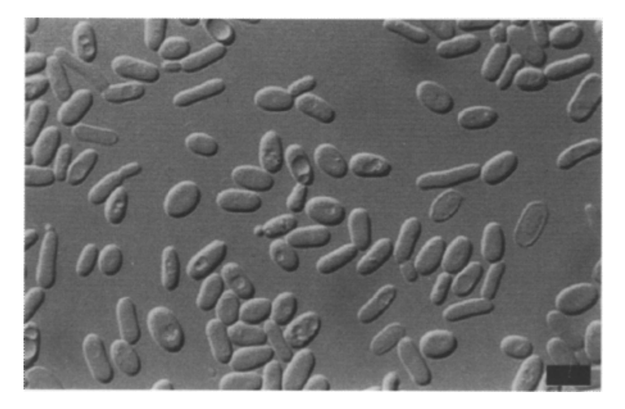


Fig. 219. *C. bombicola*, CBS 7267. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+/l	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+/l
Raffinose	–/l	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	l
L-Arabinose	–	Citrate	–/l
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	l
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	l
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 35°C	w/–
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (F.-L. Lee et al. 1993).

Mol% G+C: 49.8, CBS 6009 (T_m: Stenderup et al. 1972); 46.6, CBS 6009 (HPLC: F.-L. Lee et al. 1993).

Origin of the strains studied: CBS 6009, honey of

bumble bee (*Bombus* sp.); CBS 7267, concentrated grape juice, South Africa.

Type strain: CBS 6009.

64.22. *Candida buinensis* Soneda & S. Uchida (1971)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spheroidal to ovoidal and cylindroidal, (2.0–4.0)×(3.5–6.0) µm, and occur singly, in pairs, clusters and short chains.

Dalmau plate culture on corn meal agar: After 5 days at 25°C, pseudohyphae consist of branched chains of elongate cells with blastoconidia. Aerobic growth is white, smooth, creamy, soft and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+/l	Ethanol	+
Sucrose	+	Glycerol	l
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–/l
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–/l	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–/l	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	n
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 31.5, CBS 6796 (T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 6796, tree fern, Buin.

Type strain: CBS 6796.

64.23. *Candida butyri* Nakase (1971b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose, ovoidal to elongate, (3.6–5.7)×(3.6–7.2) µm, and occur singly, in pairs, clusters and chains. Pseudohyphae may be present.

Dalmau plate culture on corn meal agar: After 3 days at 25°C, abundant pseudohyphae consist of ramified chains of cells bearing groups of blastoconidia in verticils. Aerobic growth is white to cream-colored, shiny, smooth with some wrinkled areas and soft. The border is fringed.

Fermentation:

Glucose	+/w	Lactose	–
Galactose	w/–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+/l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+/l
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	w/–
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 34.4, 34.9, 2 strains (T_m: Nakase 1971b); 36.1, CBS 2226 (T_m: von Arx 1979a).

Origin of the strains studied: CBS 2226, abscess at site of penicillin injection; CBS 6421, butter.

Type strain: CBS 6421.

Comments: Kurtzman and Robnett (1997) found *C. aaseri* and *C. butyri* to have identical sequences in

a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.24. *Candida cantarellii* (van der Walt & van Kerken) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:
Torulopsis cantarellii van der Walt & van Kerken (1961b)
Torulopsis vinacea Ohara, Nonomura & Yamazaki (1964)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ellipsoidal, (3.0–4.0)×(4.0–8.0) µm, single, in pairs and small groups.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is cream-colored, soft, dull and wrinkled.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	–/l	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	–/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–/l
Soluble starch	–	DL-Lactate	–/l
D-Xylose	–/l	Succinate	+
L-Arabinose	–	Citrate	–/l
D-Arabinose	–	Inositol	–
D-Ribose	+/l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–/l
D-Glucuronate	–	10% NaCl/5% glucose	+/w
Xylitol	+	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	+
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	+/l
Cadaverine	–	Growth at 30°C	+
Creatinine	–	Growth at 35°C	v
L-Lysine	+/w	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 41.9, CBS 4878 (BD: Meyer et al. 1984).

Origin of the strains studied: CBS 4878, grape must in South Africa; CBS 5383, CBS 5445, CBS 5654, grape must in Japan.

Type strain: CBS 4878.

64.25. *Candida caseinolytica* Phaff, Starmer, Lachance & Ganter (1994)

This species was not included in our studies because of the time of its publication. Therefore, the description here is taken from the original description (Phaff et al. 1994).

Growth in YM broth: After 3 days at 30°C, the cells are ovoidal to short cylindroidal, (1.3–2.5)×(2.5–5.0) µm, and occur singly, in pairs or in short chains.

Growth on YM agar: After 3 days, the streak culture is white, smooth, glossy, butyrous, convex and slow growing.

Dalmau plate culture on corn meal agar: After 10 days, no pseudohyphae or true hyphae are produced.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–/l	Ethanol	+
Sucrose	–/l	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	l
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	–
5-Keto-D-gluconate	+	0.1% Cycloheximide	+
Saccharate	n	Glucono-δ-lactone gluconate	+/w
D-Glucuronate	n	Amino acid-free medium	+
Xylitol	n	Gelatin hydrolyzates	–
L-Arabinitol	n	Casein hydrolysis at pH 6.5–7.0	+
Arbutin	n	Lipolytic activity	w
Propane 1,2 diol	n	YM with 5% NaCl	+
Butane 2,3 diol	n	YM with 7.5% NaCl	–
Nitrite	–	Growth with triterpene glycosides at 25°C	+/w
Cadaverine	+	Growth with triterpene glycosides at 37°C	+
Creatinine	–	Growth at 37°C	+
L-Lysine	+	Growth at 42°C	v
Ethylamine	+	Growth at 45°C	v
D-Glucosamine (N)[1]	–		
50% Glucose	–		
10% NaCl/5% glucose	n		
Starch formation	–		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.
Mol% G+C: 46.4–47.4, 8 strains; 46.9, type strain (BD: Phaff et al. 1994).

Origin of the strains studied: Cacti (*Opuntia* spp. and *Stenocereus* spp.) in Arizona, Texas, Mexico, Hawaii, and Argentina (36 strains); Myrtillocactus cochal, Mexico (2); *Drosophila mojavensis* in Mexico (1).

Type strain: CBS 7781 from *Opuntia phaeacantha*, Santa Rita Mountains, Arizona.

Comments: This species was isolated from necrotic tissues of various cactus species in various locations. Its unique property is its exceptionally strong ability to hydrolyze casein at pH 6.5. In addition, the cells are very small. The type strain was described as having the ability to assimilate xylose, gluconate, glucono-δ-lactone, sorbose (latently) and to grow at 42° and 45°C.

64.26. *Candida castellii* (Capriotti) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis castellii Capriotti (1961d)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to cylindrical, (2.0–3.0)×(3.0–7.0) μm, single and in pairs (Fig. 220).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, no pseudohyphae are present. Aerobic growth is white to cream-colored, soft, smooth, glistening and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–/l
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–/l	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	–
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	l
Xylitol	–	10% NaCl/5% glucose	l
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	–	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.
Mol% G+C: 43.3, CBS 4332 (T_m: Meyer et al. 1984).

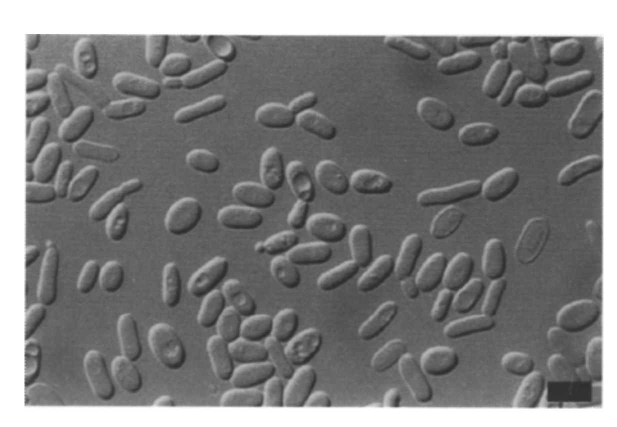


Fig. 220. *C. castellii*, CBS 4232. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Origin of the strain studied: CBS 4332, soil, Finland.

Type strain: CBS 4332.

Comments: *Candida castellii* closely resembles *C. glabrata* but can be distinguished from it by requiring inositol but not pyridoxine as a vitamin for growth.

64.27. *Candida castrensis* C. Ramírez & A. González (1984a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 20°C, the cells are elongate, (1.5–2.5)×(6.0–15.0) μm, single and in pairs (Fig. 221).

Dalmau plate culture on corn meal agar: After 7 days at 22°C, pseudohyphae consist of branched chains of elongate cells with pyriform blastoconidia; septate hyphae are also present. Aerobic growth is white, dull, and smooth with a mycelial border.

Fermentation: absent.



Fig. 221. *C. castrensis*, CBS 8172. After 3 days in glucose–yeast extract–peptone broth at 20°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	l	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	l	Inositol	+
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	l	10% NaCl/5% glucose	–
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	v	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	l
Cadaverine	+	Growth at 25°C	w
Creatinine	–	Growth at 30°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 41.7, CBS 8172 (T_m: Meyer, unpublished data).

Origin of the strain studied: CBS 8172, from rotten wood, Chile.

Type strain: CBS 8172.

Comments: The strain examined grows poorly at 25°C, consequently the assimilation test cultures were incubated at 20°C. Kurtzman and Robnett (1997) found *C. paludigena* and *C. castrensis* to differ by only two nucleotides in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.28. *Candida catenulata* Diddens & Lodder (1942)

Synonyms:

Blastodendrion brumptii Guerra (1935)
Candida brumptii (Guerra) Langeron & Guerra (1938)
Mycotorula brumptii (Guerra) Krasil'nikov (1954c)
Candida ravautii Langeron & Guerra (1938)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to cylindroidal, (1.5–4.5)×(4–12) µm, single, in pairs and chains (Fig. 222).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of chains of ovoidal or cylindroidal cells, and sometimes bear small verticils of ovoidal blastoconidia. Aerobic growth is grayish to cream-colored, somewhat dull, soft and wrinkled.

Fermentation:

Glucose	v	Lactose	–
Galactose	–/s	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	+/l
Trehalose	+/l	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+/l
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–/l
Soluble starch	v	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

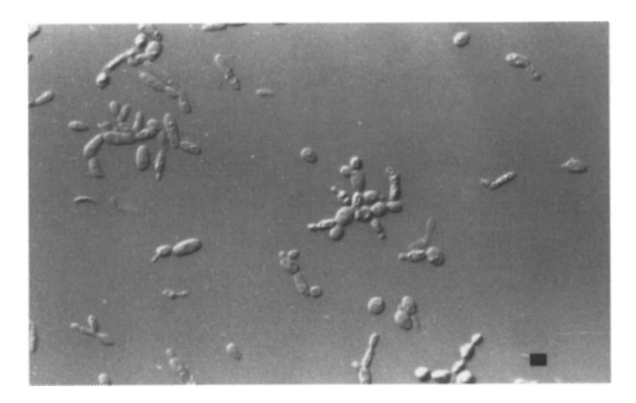


Fig. 222. *C. catenulata*, CBS 6174. After 7 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Ethylamine	v
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+/l
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	v	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–/l
Propane 1,2 diol	v	Pyridoxine-free	+
Butane 2,3 diol	+/l	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	+/l
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 54.5, CBS 565; 54.1, CBS 564 (T_m: Stenderup and Bak 1968); 53.2, C-140 (Langeron #495); 53.2, CBS 1904; 53.4, UCD-FS&T 60-60 (T_m: Meyer and Phaff 1972); 53.2, 54.4, IFO 0745 (CBS 565); 54.4, IFO 0744 (CBS 1904) (T_m: Nakase and Komagata 1971f).

Origin of the strains studied: CBS 564, case of perleche (*C. brumptii*); CBS 565, from dysentery patient; CBS 1904, hyperkeratinic foot (*C. ravautii*); CBS 2014, chicken's gut; CBS 2743; CBS 6145, skin; CBS 6174, mastitis milk; CBS 6824; CBS 7135, CBS 7230, soil.

Type strain: CBS 565.

Comments: The conspecificity of *C. catenulata*, *C. brumptii* and *C. ravautii* was established by DNA reassociation experiments (Simione and Meyer 1978).

64.29. *Candida chilensis* Grinbergs & Yarrow (1970a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are short ovoidal to elongate, (3.6–5.8)×(4.3–7.2) µm, single, in pairs and small groups. After one month a creeping pellicle is present and the cell suspension is mucoid.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, branched chains of cylindrical cells give a wavy appearance. A few to many subglobose to ovoidal blastoconidia appear individually or in clusters. Some septate hyphae are evident. Aerobic growth is cream-colored to beige, shiny, smooth and butyrous. The margin is entire to slightly fringed.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	+	Growth at 25°C	+
Cadaverine	+	Growth at 30°C	w/–
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 44.0, CBS 5719 (BD: Meyer et al. 1984); 42.7, CBS 5719 (T_m: Stenderup et al. 1972).

Origin of the strains studied: CBS 5719, CBS 5720, from rotten wood, Chile.

Type strain: CBS 5719.

64.30. *Candida chiropterorum* Grose & Marinkelle (1968)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoid, (2.2–2.8)×(4.4–7.0) µm, and occur in branched chains and small branched clusters. Few single cells or budding cells are present. After one month a thick sediment is evident.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, branched septate hyphae with small chains or clusters of blastoconidia are present. Aerobic growth is white to cream-colored and dry in appearance. Filamentous outgrowths give a feathery, 'fuzzy' appearance and the margin is fringed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	l	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	l
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	+
D-Arabinose	l	Inositol	+
D-Ribose	l	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	n
Saccharate	–	50% Glucose	+
D-Glucuronate	+	10% NaCl/5% glucose	+
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	+
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	l
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 48.0, CBS 6064 (T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 6064, liver of bat (*Mormoops megalophylla*).

Type strain: CBS 6064.

64.31. *Candida coipomoensis* C. Ramírez & A. González (1984f)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.0–4.0)×(3.0–4.5) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, well-developed pseudohyphae are present. Aerobic growth is white, smooth, and butyrous with a fringed border.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	l	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–/l	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	l
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 32.0, CBS 8178 (T_m: Meyer, unpublished data).

Origin of the strain studied: CBS 8178, rotten wood, Chile.

Type strain: CBS 8178.

64.32. *Candida conglobata* (Redaelli) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)

Synonyms:

Cryptococcus conglobatus (Redaelli) Pollacci & Nannizzi (Nannizzi 1934)

Torulopsis conglobata Redaelli (1925)

Candida conglobata (Redaelli) van Uden & H.R. Buckley (1970) nom. inval.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to cylindrical, (3.0–5.0)×(5.0–20.0) μm, single and in chains.

Dalmau plate culture on corn meal agar: Pseudohyphae consist of long, branched chains of cylindrical cells, usually with few blastoconidia. Aerobic growth is cream-colored and delicately wrinkled.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+/l	Inositol	–
D-Ribose	+/l	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	n
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 39.2–40.9, CBS 2018, CBS 5808 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 2018, tubercular lung; CBS 2019, probably human origin; CBS 5808, bark of tree, Chile.

Type strain: CBS 2018.

64.33. *Candida cylindracea* K. Yamada & Machida ex S.A. Meyer & Yarrow (1998)

Synonym:

Candida cylindracea K. Yamada & Machida (1962) nom. inval.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are elongate, (2.0–5.0)×(4.0–12.0) μm, in pairs and chains. Growth is predominantly pseudomycelial with very few single cells present. A thick wrinkled pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of elongate cells with little branching and few blastoconidia. A wavy appearance is evident. Aerobic growth is white, smooth and lacy, crater-like and heavily fringed with mycelium.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	–
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 62.7, CBS 6330 (T_m: Meyer and Yarrow 1998).

Origin of the strain studied: CBS 6330, soil.

Type strain: CBS 6330.

Comments: This species, which was previously placed in synonymy with *Candida zeylanoides*, has been restored because of the significant difference of mol% G+C. In addition, the rRNA sequence comparison of these two species supports this separation (Kurtzman, personal communication).

64.34. *Candida dendrica* (van der Walt, van der Klift & D.B. Scott) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis dendrica van der Walt, van der Klift & D.B. Scott (van der Walt et al. 1971b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to short-ovoidal, some cylindrical, (2.0–5.5)×(3.0–6.5) μm, and occur singly, in pairs, short chains and clusters.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is smooth, shiny, cream-colored with an entire to somewhat irregular margin.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	n
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	–	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 42.0, CBS 6151 (T_m: Stenderup et al. 1972).

Origin of the strains studied: CBS 6151, 6227, frass of beetle larvae, South Africa.

Type strain: CBS 6151.

64.35. *Candida dendronema* van der Walt, van der Klift & D.B. Scott (van der Walt et al. 1971a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are various shapes: globose, ovoid, elongate, teardrop, and triangular to cylindrical, (2.2–5.1)×(3.6–5.7) µm, and occur singly, in pairs and in small clusters and short chains. Pseudohyphae are present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, well-developed pseudohyphae are present. The blastoconidia are curved, globose, triangular or teardrop shaped and arranged in grape-like clusters. Aerobic growth is off-white, dry, raised, wrinkled and with a fringed border.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–/s	Trehalose	s
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	+	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	l	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	l
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	w/–
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 38.9, CBS 6270 (T_m: Meyer et al. 1984); 38.5, CBS 6270; 38.0, CBS 6271 (HPLC: C.-F. Lee et al. 1993).

Origin of the strains studied: CBS 6270, CBS 6271, frass of beetle larvae, South Africa.

Type strain: CBS 6270.

64.36. *Candida diddensiae* (Phaff, Mrak & Williams) Fell & S.A. Meyer ex S.A. Meyer & Ahearn (1983)

Synonyms:

Trichosporon diddensiae Phaff, Mrak & Williams (1952)
Torulopsis saccharini Santa María (1959b)
Candida diddensiae (Phaff, Mrak & Williams) Fell & S.A. Meyer (1967) nom. inval.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose, pear-shaped to cylindroidal, (2.3–4.6)×(2.5–5.7) µm, and occur singly, in pairs, short chains and clusters. After one month a thin filmy pellicle and sediment are present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindroidal cells with verticils of ovoidal blastoconidia

giving a wavy appearance. Aerobic growth is off-white to cream-colored, lacy to wrinkled with a mycelial border.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–/s	Trehalose	s
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–/l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	l
Melezitose	+	Salicin	+/l
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+/l	Inositol	–
D-Ribose	+/l	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+/s
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	v

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 38.8; 38.3; 36.1, CBS 2214 (T_m: Meyer and Phaff 1972; T_m: Daniel 1983; HPLC: C.-F. Lee et al. 1993, respectively); 37.3, AJ 5108 (*T. saccharini*) (T_m: Nakase and Komagata 1971e).

Origin of the strains studied: CBS 2214, shrimp, USA; CBS 4514, sugar, Spain.

Type strain: CBS 2214.

Comments: DNA reassociation studies (Meyer and Simione 1978) demonstrated a high degree of relatedness between the type strain of *Torulopsis saccharini* and *C. diddensiae*. Other species (*C. atmosphaerica*, *C. polymorpha* and *Trichosporon atlanticum*) that had been considered synonyms of *C. diddensiae* (van Uden and Buckley 1970) were found to have low DNA relatedness and were restored to the species level (Meyer et al. 1984). C.-F. Lee et al. (1993) showed low DNA relatedness between *C. diddensiae* and *C. dendronema*, and *C. diddensiae* and *C. terebra*. Kurtzman and Robnett (1997) found *C. diddensiae* and *C. naeodendra* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.37. *Candida diversa* Y. Ohara, Nonomura & Yunome ex van Uden & H.R. Buckley (1970)

Synonyms:

Candida fimetaria Soneda var. *diversa* Ohara, Nonomura & Yunome (1960b)

Torulopsis arnaudii Capriotti & Fatichenti (1969)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoid to short cylindrical, (1.5–4.5)×(5.0–8.0) μm, and occur singly and in short chains.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, primitive pseudohyphae consisting of branched chains of cylindrical cells with clusters of blastoconidia may be present. Aerobic growth is white, smooth, and creamy with an entire border.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–/l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	n
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	w/–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (Yamada and Kondo 1972a).

Mol% G+C: 34.4, AJ 4648; 34.9, AJ 5367 (T_m: Nakase and Komagata 1971f); 36.0, CBS 6102; 36.5, CBS 6103; 35.1, 36.6, CBS 4074 (T_m: Suzuki et al. 1994; BD: Meyer et al. 1984).

Origin of the strains studied: CBS 4074, grape must, Japan; CBS 6102, CBS 6103, grape must, Italy.

Type strain: CBS 4074.

Comments: Suzuki et al. (1994) described a new species, *C. stellimalicola*, that has physiological and morphological properties similar to *C. diversa, C. karawaiewii* and *C. silvae*. It is differentiated from *C. diversa* on its ability to assimilate D-arabinose and DL-lactic acid, its inability to assimilate ribitol and the lack of fermentation. It differs from *C. karawaiewii* by its ability to assimilate L-lysine and from *C. silvae* on a vitamin requirement. DNA reassociation, cell surface antigens and proton magnetic resonance spectra of cell wall mannans were employed to differentiate these species.

64.38. *Candida drimydis* C. Ramírez & A. González (1984j)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.5–6.0)×(2.8–6.9) µm, single and in pairs. A sediment is present (Fig. 223). After 30 days a ring is present.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, no pseudohyphae are present. Aerobic growth is white, smooth, and waxy with an undulating margin.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	l
Maltose	+	Erythritol	–
Cellobiose	l	Ribitol	l
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	l
Raffinose	–	α-Methyl-D-glucoside	l
Melezitose	–	Salicin	l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	l	Succinate	+
L-Arabinose	–	Citrate	l
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	l	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	l	Urease	–
Propane 1,2 diol	l	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 43.4, CBS 8185 (T_m: Tengku Zainal Mulok 1988).

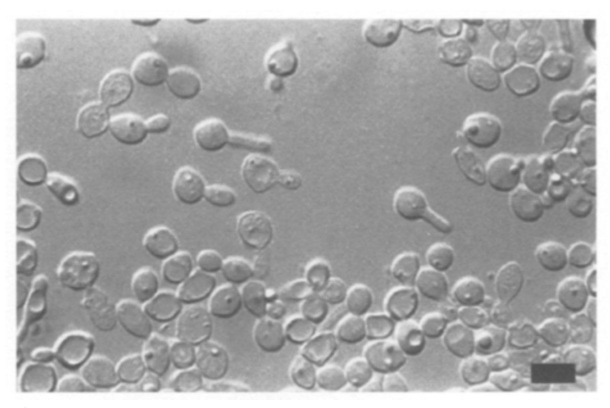


Fig. 223. *C. drimydis*, CBS 8185. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Origin of the strain studied: CBS 8185, rotten wood (*Drimys winteri*), Chile.

Type strain: CBS 8185.

Comments: Kurtzman and Robnett (1997) found *C. petrohuensis, C. ancudensis* and *C. drimydis* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, thus indicating the three taxa to be conspecific.

64.39. *Candida edax* van der Walt & E.E. Nel (1968)

See *Stephanoascus smithiae*: p. 402

Comments: Giménez-Jurado et al. (1994) demonstrated *C. edax* to be the anamorph of *Stephanoascus smithiae* from mating reactions and high nuclear DNA complementarity.

64.40. *Candida entomophila* D.B. Scott, van der Walt & van der Klift (van der Walt et al. 1971a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spheroidal, ovoidal, long ovoidal, cylindrical, teardrop to somewhat triangular and occur singly, with buds, and in chains. The cell size differs in the two strains which measure (2.8–4.2)×(7.0–8.4) µm and (5.6–7.0)×(14.0–21.0) µm (Fig. 224). Spheroidal to ellipsoidal asexual endospores are formed in hyphal strands.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae are abundant and consist of long straight chains of cylindrical cells with little branching and often without blastoconidia. When blastoconidia are present, they are ovoidal to triangular and occur singly, in small clusters and short chains. Septate hyphae are also present. Aerobic growth is white to cream-colored, semi-shiny to dull, and smooth with wrinkled areas. The margin is fringed.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	s
Sucrose	+	Trehalose	+
Maltose	–		

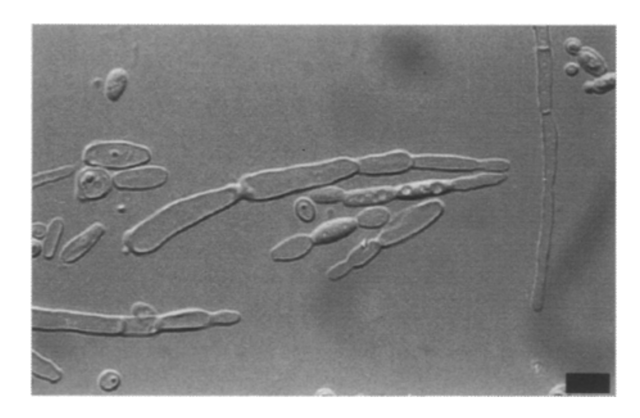


Fig. 224. *C. entomophila*, CBS 6160. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–/l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+/l	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	v
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	w/–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 56.3, CBS 6160 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 6159, CBS 6160, tunnels of beetles in trees, South Africa.

Type strain: CBS 6160.

64.41. *Candida ergastensis* Santa María (1971)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to elongate and cylindroidal, (2.0–4.0) × (4.5–15.0) µm, single and in chains.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae are abundant and consist of chains of cylindroidal cells, often long and slender, with oval blastoconidia. Septate hyphae may also be present. Aerobic growth is white to cream-colored, somewhat shiny, and folded with a fringed margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	l	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	l	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	–/l	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	–
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 36.5, CBS 6248 (BD: Meyer et al. 1984).

Origin of the strain studied: CBS 6248, bark beetles (*Ergastes faber*), Spain.

Type strain: CBS 6248.

64.42. *Candida ernobii* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis ernobii Lodder & Kreger-van Rij (1952)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose, (3.0–5.0) × (3.0–6.0) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent or consist

of short chains of cells. Aerobic growth is off-white to cream-colored, soft, smooth or slightly wrinkled.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	l
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+/l
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	l
L-Arabinose	l	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	l	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	+	Biotin-free	+
Propane 1,2 diol	–/l	Pyridoxine-free	+
Butane 2,3 diol	–/l	0.01% Cycloheximide	+
Nitrite	+	0.1% Cycloheximide	v
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	w
L-Lysine	+	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 8 (Yamada and Kondo 1972a).

Mol% G+C: 36.1, CBS 1737 (T_{m}: Nakase and Komagata 1971e).

Origin of strain studied: CBS 1737, bark beetles (*Ernobius mollis*).

Type strain: CBS 1737.

Comments: Kurtzman and Robnett (unpublished data) found *C. ernobii* and *C. karawaiewii* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific. Furthermore, the sequence of *Pichia holstii* differed by only two nucleotides suggesting that it is either the teleomorph or a sibling species.

64.43. *Candida etchellsii* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Brettanomyces sphaericus Etchells & T.A. Bell (1950b)
Torulopsis citrus Recca & Mrak (1952)
Torulopsis etchellsii Lodder & Kreger-van Rij (1952)
Torulopsis halonitratophila Onishi ex van Uden & Vidal-Leiria (1970)
Candida halonitratophila (Onishi ex van Uden & Vidal-Leiria) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Torulopsis nodaensis Onishi (1957) nom. nud.
Candida nodaensis Yarrow & S.A. Meyer (1978)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.0–4.0)×(3.0–5.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is off-white to cream-colored, shiny or somewhat dull, and smooth with a entire margin.

Fermentation:

Glucose	v	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	–	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–/l
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	v	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	v
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	+	0.01% Cycloheximide	–
Cadaverine	v	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 52.0–52.7, 4 strains (T_{m}: Nakase and Komagata 1971d); 52.4, CBS 6268 (T_{m}: Meyer et al. 1984); 53.0, CBS 3094 (T_{m}: Yarrow and Meyer 1978); 52.0, CBS 1750; 52.4, JCM 5956; 51.4, CBS 5240; 52.0, CBS 3094 (T_{m}: Suzuki et al. 1992).

Origin of the strains studied: CBS 1750, CBS 1751, fermenting cucumber brine, USA; CBS 2853, sputum, Netherlands; CBS 2854, unknown, probably of human origin; CBS 2987, concentrated lemon juice (type strain

of *Torulopsis citrus*); CBS 3094, soy mash, Japan (type strain of *C. nodaensis*); CBS 4852, sputum; CBS 5008, sugar, Mauritius; CBS 5240, soy mash, Japan (type strain of *C. halonitratophila*); CBS 6268, soy mash, Japan; CBS 8147, sausages, Netherlands.

Type strain: CBS 1750.

Comments: Suzuki et al. (1992) did taxonomic studies of halophilic and halotolerant *Candida* species found in the ripening process of soy sauce mash. They employed several criteria: proton magnetic resonance spectra of cell wall galactomannans, DNA base composition, electrophoretic patterns of enzymes and DNA–DNA hybridization. The results demonstrated that *C. halonitratophila* and *C. nodaensis* are conspecific with *C. etchellsii*. F.-L. Lee et al. (1992) employed DNA reassociations on these same species and reported their conspecificity.

64.44. *Candida ethanolica* Rybářová, Štros & Kocková-Kratochvílová (1980)

Synonyms:

Torulopsis ethanolitolerans Rybářová, Štros & Kocková-Kratochvílová (1981)

Torulopsis ethanolitolerans Rybářová, Štros & Kocková-Kratochvílová var. *minor* Rybářová, Štros & Kocková-Kratochvílová (1981)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (5.0–7.0)×(6.0–10.0) µm, single and in pairs. After one month, a thin pellicle is present and growth is evident on the glass surface.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells. Aerobic growth is white to cream-colored, smooth to delicately wrinkled, lobed and entire.

Fermentation:

Glucose	s/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–/l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+/l
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	+
Xylitol	–	10% NaCl/5% glucose	n
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 29.3, CBS 6753 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 6753 (ATCC 14927) source unknown; CBS 8041, CBS 8084 (type of *Torulopsis ethanolitolerans*); CBS 8085 (type of *Torulopsis ethanolitolerans* var. *minor*), industrial fodder yeasts from Czechoslovakia.

Type strain: CBS 8041.

Comments: Kurtzman and Robnett (unpublished data) found *C. ethanolica* and *Pichia deserticola* to differ by only two nucleotides in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be either conspecific or sibling species.

64.45. *Candida famata* (Harrison) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

This species has two varieties:

***Candida famata* (Harrison) S.A. Meyer & Yarrow (Yarrow and Meyer 1978) var. *famata* (1985)**

See *Debaryomyces hansenii* var. *hansenii*: p. 161

***Candida famata* var. *flareri* (Ciferri & Redaelli) Nakase & Suzuki (1985b)**

See *Debaryomyces hansenii* var. *fabryi*: p. 162.

Comments: Nakase and Suzuki (1985a,b) made a thorough investigation of *Debaryomyces hansenii*. Various strains of *Candida* species that at one time had been recognized as anamorphs of *D. hansenii* were included. DNA base composition, DNA reassociation, PMR spectra, isoenzymes, and serology were employed in these studies.

64.46. *Candida fennica* (Sonck & Yarrow) S.A. Meyer & Ahearn (1983)

Synonyms:

Trichosporon fennicum Sonck & Yarrow (1969)

Trichosporon melibiosaceum D.B. Scott & van der Walt (1970b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, cells are globose or ovoidal to elongate, (2.2–6.5)×(2.9–6.5) µm. Pseudohyphae and true hyphae are also present. After one month the sediment is clumpy and the broth appears viscous.

Dalmau plate culture on corn meal agar: After 7 days

at 25°C, pseudohyphae and true hyphae are present. Long wavy, branched chains of cells with globose to ovoidal blastoconidia are abundant as well as true hyphae which have knobby protuberances along the hyphal strands. Aerobic growth is white to off-white, folded, wrinkled, dull, dry to powdery, with a mycelial border.

Fermentation:

Glucose	+	Lactose	s/–
Galactose	s	Raffinose	s/–
Sucrose	s	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–/l	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	+/l
Melezitose	v	Salicin	+/l
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+/l	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	v
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	+/l

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+/l	10% NaCl/5% glucose	+
L-Arabinitol	v	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	s
Butane 2,3 diol	v	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	w/–
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 34.7, CBS 5928; 37.9, CBS 5999 (T_m: Guého et al. 1984).

Origin of the strains studied: CBS 5928, cat's anus, Finland; CBS 5999, insect frass from tree, South Africa; CBS 6027, CBS 6028, poultry or birch trees, Finland; CBS 6087, frass from tree, South Africa (type of *Trichosporon melibiosaceum*).

Type strain: CBS 5928.

Comments: This species closely resembles *Pichia burtonii*, but no mating has been observed.

64.47. *Candida fermenticarens* van der Walt & P.B. Baker (1978)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.5–4.0)×(3.0–5.0) µm, and are single or in pairs (Fig. 225).

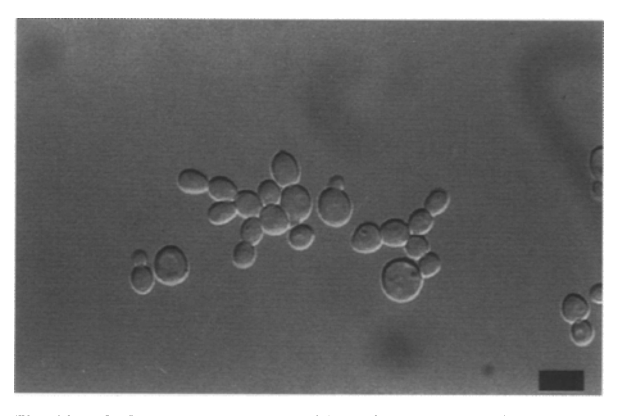


Fig. 225. *C. fermenticarens*, CBS 7040. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongated cells, sometimes with verticils of ovoidal blastoconidia. Aerobic growth is white to cream-colored, smooth and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	l	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	l
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	l	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	–
Creatinine	–		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strain studied: CBS 7040, lichen, South Africa.

Type strain: CBS 7040.

64.48. *Candida floricola* Tokuoka, Ishitani, S. Goto & Komagata (1987)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.0–3.0)×(3.0–4.5) μm, single and in pairs (Fig. 226).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae are not present. Aerobic growth is white, smooth, butryous and entire.

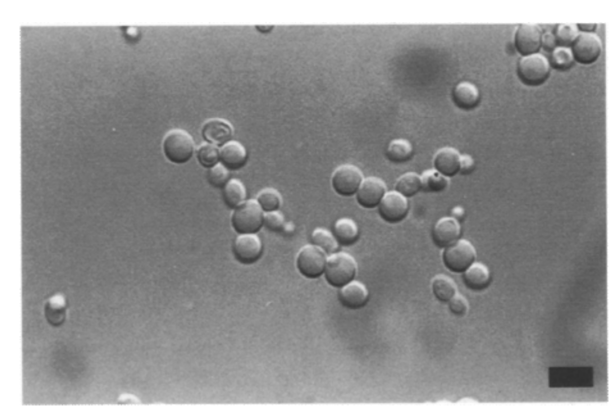


Fig. 226. *C. floricola*, CBS 7289. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	+/l		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+/l
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–/l
Soluble starch	–	DL-Lactate	–
D-Xylose	–/l	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	–/l	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Tokuoka et al. 1987).

Mol% G+C: 51.7, CBS 7289 (T_m: Tokuoka et al. 1987); 51.7, CBS 7289 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 7289, CBS 7290, dandelion (*Taraxacum platycarpum*), Japan.

Type strain: CBS 7289.

64.49. *Candida fluviatilis* Hedrick (1976)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (1.5–4.0)×(3.0–5.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent or primitive, consisting of short branched chains of ovoidal cells. Aerobic growth is white to cream-colored, smooth, glossy to dull and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–/l		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+/l
Soluble starch	+	DL-Lactate	+/l
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	+/l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	l	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 39.8, CBS 6776; 40.0, CBS 6775 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 6775, CBS 6776, polluted river, Indiana, USA.

Type strain: CBS 6776.

64.50. *Candida freyschussii* H.R. Buckley & van Uden (1968)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (3.0–5.0)×(5.0–13.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of somewhat elongated cells, sometimes with ovoidal blastoconidia. Aerobic growth is whitish to cream-colored, soft, smooth and glistening.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	s/–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	v
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 45.3, type strain (BD: Meyer et al. 1984); 43.8, 2 strains (BD: Holzschu et al. 1979).

Origin of the strains studied: CBS 2161, CBS 2162, wood pulp, Sweden.

Type strain: CBS 2162.

Comments: The type strain produces hat-shaped ascospores on 5% Difco malt agar. Kurtzman (personal communication) also observed very small ascospores in the type strain. However, the ascospores were not viable. More studies need to be performed before decisions can be made about the teleomorphic stage of this organism.

64.51. *Candida friedrichii* van Uden & Windisch (1968)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to cylindroidal, (1.5–2.5)×(5.0–12.0) μm, single and in short chains (Fig. 227). A thin pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of chains of cylindroidal cells, sometimes bearing short chains of ovoidal blastoconidia. Some pseudohyphae bear many blastoconidia whereas others have few. Aerobic growth is white to cream-colored, soft, wrinkled with a frosted appearance and with a fringed border.

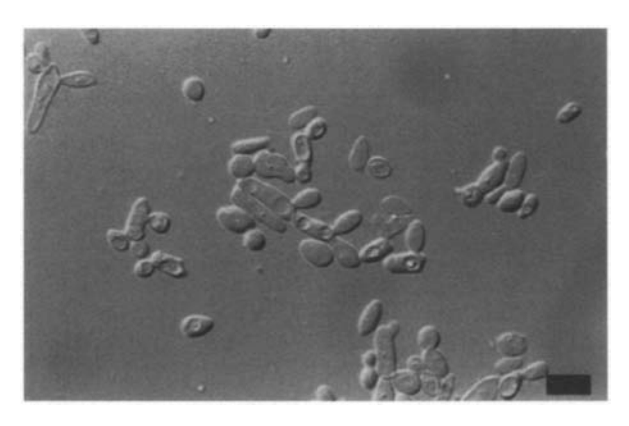


Fig. 227. *C. friedrichii*, CBS 6937. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	s/–	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	w/–
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 32.9–33.7, 2 strains (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 4114, D-glucitol solution, Germany; CBS 6937, cheese, France.

Type strain: CBS 4114.

64.52. *Candida fructus* (Nakase) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis fructus Nakase (1971a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.4–5.6)×(3.4–6.7) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, primitive pseudohyphae of short chains of ovoidal cells are sparsely formed. Aerobic growth is off-white, shiny, smooth and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	n
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 48.8, CBS 6380 (T_m: Nakase 1971b).

Origin of the strain studied: CBS 6380, banana, Japan.

Type strain: CBS 6380.

Comments: Kurtzman and Robnett (1997) found *C. fructus* and *C. musae* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.53. *Candida galacta* (Golubev & Bab'eva) F.-L. Lee, C.-F. Lee, Okada, Komagata & Kozak (1993)

Synonym:

Torulopsis apis var. *galacta* Golubev & Bab'eva (1977)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spheroidal, (1.5–3.5) μm, and occur singly, in pairs, in small chains and in clusters.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is white to light beige in color, smooth, creamy, soft, and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	l	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	l
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	+
Xylitol	–	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 8 (F.-L. Lee et al. 1993).

Mol% G+C: 50.2, CBS 6939 (HPLC: F.-L. Lee et al. 1993); 49.8, CBS 6939 (T_m: Meyer, unpublished data).

Origin of the strain studied: CBS 6939, from cocoons of ants.

Type strain: CBS 6939.

Comments: F.-L. Lee et al. (1993) established this yeast as a distinct species. Formerly, it was classified as *C. apis* var. *galacta*. *C. galacta* and *C. apis* were found to differ in mol% G+C and Co-Q, and also demonstrated a low degree of DNA reassociation.

64.54. *Candida geochares* (van der Walt, E. Johannsen & Yarrow) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis geochares van der Walt, E. Johannsen & Yarrow (1978)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (3.0–5.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is white, butyrous, smooth and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	s	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	l	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	l	Ribitol	l
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	l
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	l	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	+
Xylitol	l	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	w

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 54.1, CBS 6870 (T_m: van der Walt et al. 1978).

Origin of the strain studied: CBS 6870, soil, South Africa.

Type strain: CBS 6870.

64.55. *Candida glabrata* (Anderson) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Cryptococcus glabratus Anderson (1917)
Torulopsis glabrata (Anderson) Lodder & de Vries (1938)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.5–4.0)×(3.0–6.0) μm, single and in pairs (Fig. 228).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent or consist of a few short chains of ovoidal cells. Aerobic growth is white, smooth, butyrous and entire.

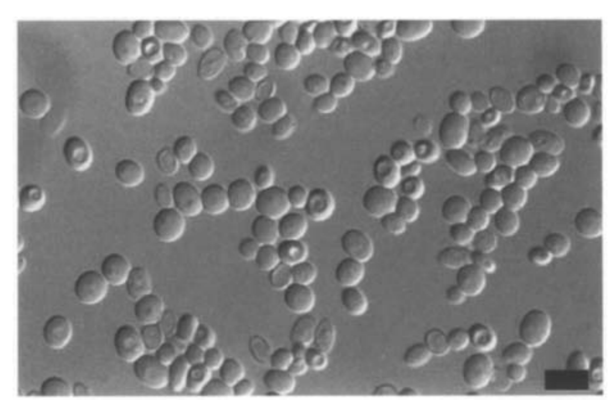


Fig. 228. *C. glabrata*, CBS 7307. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	–	Glycerol	+/l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	v	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	L-Lysine	w/–
5-Keto-D-gluconate	–	Ethylamine	–
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	v
Xylitol	–	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	v
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 6 (Yamada and Kondo 1972a).

Mol% G + C: 39.6–40.2, 5 strains (Mendonça-Hagler and Phaff 1975); 38.5–39.5, 2 strains (T_m: Nakase and Komagata 1971d).

Origin of the strains studied: CBS 138, feces; CBS 858, CBS 859, unknown, probably human; CBS 861, CBS 862, mouth; CBS 863, urine; CBS 1518, baker's yeast; CBS 2192, sorghum malt; CBS 2498, culture medium with pH of 2; CBS 2661, calf fetus; CBS 4331, fermenting passion-fruit juice; CBS 5278, (IGC 2990) unknown; CBS 6144, vagina; CBS 7307, sputum, New Zealand.

Type strain: CBS 138.

Comments: For the purposes of routine identification, *C. glabrata* can be distinguished from *C. castellii* by its vitamin requirements. *C. glabrata* requires pyridoxine for growth, but not inositol.

64.56. *Candida glaebosa* Komagata & Nakase (1965)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–3.0) × (3.0–6.0) µm, single, in pairs and dense clusters (Fig. 229).

Dalmau plate culture on corn meal agar: After 4 days at 25°C, pseudohyphae consist of dense chains of elongated cells. Aerobic growth is white, smooth, butyrous and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+/l	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	v
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

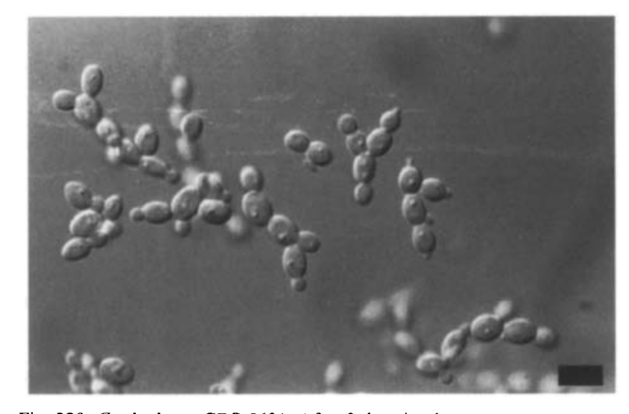


Fig. 229. *C. glaebosa*, CBS 5691. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	+
Saccharate	n	50% Glucose	–
d-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	+	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 42.9, 42.4, CBS 5691 (T_m: Nakase and Komagata 1971f; BD: Meyer et al. 1984, respectively); 41.2–43.1, 5 strains; 42.5 CBS 5691 (T_m: Suzuki and Nakase 1993).

Origin of the strains studied: CBS 5691, frozen cuttle-fish; CBS 6715, soil.

Type strain: CBS 5691.

Comments: Suzuki and Nakase (1993) compared strains of *C. glaebosa* and *C. saitoana* as well as '*Torulopsis candida*' strains that have mol% G+C comparable to that of *C. glaebosa*. They found three groups based on DNA reassociation and electrophoretic protein profiles of eight enzymes. The three groups represented three different species, *C. glaebosa, C. saitoana* and a new species which they described as *C. pseudoglaebosa*. CBS 6715, listed above as a member of *C. glaebosa*, is the type strain of the new species. These investigators reported that it is difficult to separate the three species using the traditional physiological tests. They found that *C. pseudoglaebosa* does not assimilate D- and L-arabinose, ribitol, DL-glyceraldehyde and dihydroxyacetone, whereas *C. glaebosa* and *C. saitoana* assimilate the latter three compounds and differ in the assimilation of D-arabinose (*C. glaebosa*, +; *C. saitoana*, –) and L-arabinose (*C. saitoana*, +; *C. glaebosa*, –).

64.57. *Candida glucosophila* Tokuoka, Ishitani, S. Goto & Komagata (1987)

The species description presented here was taken from the original description (Tokuoka et al. 1987).

Growth in 25% (w/w) glucose–YM broth: After 3 days at 25°C, growth is very poor. After 7 days, the cells are globose to subglobose, (4.2–7.0)×(4.2–7.3) µm, and occur singly, in pairs, in short chains and in clusters. After one month only a sediment is present.

Growth on 25% (w/w) glucose–YM agar: After one month at 25°C, the growth is cream-colored, dull, dry, smooth and entire.

Slide culture on 25% glucose–corn meal agar: Pseudohyphae are not formed.

Fermentation (in media containing 10% w/v NaCl):

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	n
Maltose	–		

Assimilation (in media containing 10% w/v NaCl):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	w
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	w	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	L-Lysine	–
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	n
D-Glucuronate	n	50% Glucose	+
Xylitol	n	10% NaCl/5% glucose	n
L-Arabinitol	n	Starch formation	n
Arbutin	n	Urease	–
Propane 1,2 diol	n	Biotin-free	n
Butane 2,3 diol	n	Pyridoxine-free	n
Nitrite	n	0.01% Cycloheximide	–
Cadaverine	n	Growth at 37°C	+
Creatinine	n	Growth at 42°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (T_m: Tokuoka et al. 1987).

Mol% G+C: 36.6, type strain (T_m: Tokuoka et al. 1987).

Origin of the strain studied: CBS 7349, brown sugar made in Taiwan.

Type strain: CBS 7349.

Comments: The osmotic requirements of this yeast make it difficult to conduct the usual morphological and physiological studies. As indicated, 10% NaCl was used in the liquid assimilation and fermentation media and 25% glucose was used in the solid media.

64.58. *Candida gropengiesseri* (Harrison) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Torula gropengiesseri Harrison (1928)
Torulopsis gropengiesseri (Harrison) Lodder (1934)
Cryptococcus gropengiesseri (Harrison) Skinner (1950)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are long-ovoidal, (1.5–2.0)×(3.0–7.0) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is off-white to cream-colored, glistening, soft, butyrous and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	s	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+/l	Methanol	–
L-Sorbose	+	Ethanol	w/–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	v	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+/l
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–/l	Succinate	+/l
L-Arabinose	–	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	–/l	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+/l	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	+/l	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–/l
Nitrite	v	0.1% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	v
L-Lysine	+	Growth at 37°C	v

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 55.7, CBS 156 (BD: Meyer et al. 1984), 57.1, CBS 156 (T_m: Nakase and Komagata 1971d).

Origin of the strains studied: CBS 156, cocoon of cockroach (*Periplaneta orientalis*); CBS 6387, human toe; CBS 7074, citrus product.

Type strain: CBS 156.

64.59. *Candida guilliermondii* (Castellani) Berkhout (1923)

This species has two varieties:

***Candida guilliermondii* (Castellani) Berkhout var. *guilliermondii* (1952)**

See *Pichia guilliermondii*: p. 308

***Candida guilliermondii* var. *membranifaciens* (as *membranaefaciens*) Lodder & Kreger-van Rij (1952)**

See *Pichia ohmeri*: p. 329

64.60. *Candida haemulonii* (van Uden & Kolipinski) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis haemulonii van Uden & Kolipinski (1962)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose, ovoidal and ellipsoidal, (2.0–7.0)×(2.0–7.0) µm, single and in pairs. (Fig. 230). A ring or a thin pellicle may be present.

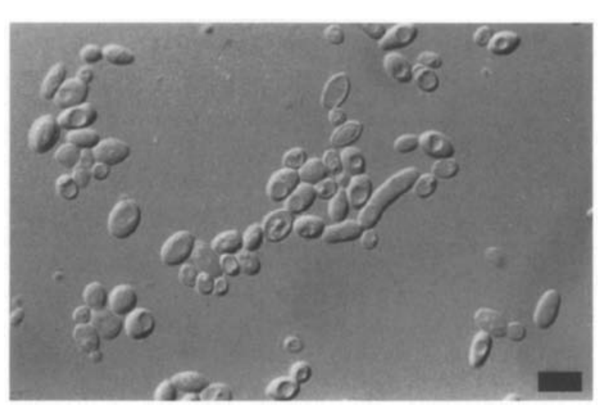


Fig. 230. *C. haemulonii*, CBS 5468. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, primitive pseudohyphae are sometimes present. Aerobic growth is off-white to light beige, smooth, and butyrous with an entire border.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	+/s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	l	Methanol	–
L-Sorbose	–	Ethanol	l
Sucrose	+	Glycerol	+/l
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	l
Trehalose	+	Galactitol	–/l
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+/l	α-Methyl-D-glucoside	–
Melezitose	+/l	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	–
D-Xylose	–/l	Succinate	+
L-Arabinose	–/l	Citrate	+/l
D-Arabinose	–/l	Inositol	–
D-Ribose	–/l	Hexadecane	+/l
L-Rhamnose	+/l	Nitrate	–
D-Glucosamine	+/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	–
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	v	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	v	Urease	–
Propane 1,2 diol	v	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 45.9–46.1, 2 strains (T_m: Nakase and Komagata 1971d); 47.8, CBS 5149 (T_m: Meyer et al. 1984); 45.4, CDC 86-041135; 46.3, CBS 5149; 47.9, CBS 7801 (T_m: Gargeya et al. 1991); 45.6–46.3, 8 strains including the type strain, 46.1 (T_m: Ribeiro 1995).

Origin of the strains studied: CBS 5149, gut of blue-striped grunt (*Haemulon sciurus*); CBS 5150, seawater, Portugal; CBS 5468, water, Florida, USA; CBS 6332, skin of captive dolphin; CBS 6590, human; CBS 8125, furniture polish.

Type strain: CBS 5149.

Comments: Lehmann et al. (1993) studied twenty-five clinical isolates that had been identified as *C. haemulonii.* These investigators used electrophoretic isoenzyme profiles, mol% G+C and DNA reassociations, as well as morphological and physiological characteristics, to examine these strains. Two distinct groups were evident and designated Group I and Group II. *C. haemulonii* type strain and eleven isolates comprised Group I. The remaining fourteen isolates made up Group II. Some physiological differences were noted between the two groups. Group I strains demonstrated negative or latent reactions on galactose, sorbose, melezitose, L-arabinose, galactitol, and α-methyl-D-glucoside, whereas the Group II strains showed positive responses on these carbon sources. Ribeiro (1995) examined the CBS strains included in *C. haemulonii* to determine if the two groups were present. DNA reassociation experiments and restriction fragment length polymorphisms showed that most of the strains were the same as the type strain (Group I) and are considered valid members of *C. haemulonii.* One strain demonstrated significant DNA reassociation with members of Group II. Group II strains will be designated a new species.

64.61. *Candida homilentoma* van der Walt & Nakase (1973)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to elongate, (2.5–3.5)×(3.0–4.5) µm, single and in pairs (Fig. 231).

Dalmau plate culture on corn meal agar: After 3 days at 25°C, pseudohyphae of long cylindrical cells and septate hyphae with few blastoconidia are present. Aerobic growth is off white, dull, and wrinkled with a mycelial border.



Fig. 231. *C. homilentoma,* CBS 6099. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–/l
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	l	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	l	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	v
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+/w
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 48.3, CBS 6099; 49.0, CBS 6312 (T_m: van der Walt and Nakase 1973).

Origin of the strains studied: CBS 6099, CBS 6312, tunnels and frass of insects infesting trees in South Africa.

Type strain: CBS 6312.

64.62. *Candida humilis* (E.E. Nel & van der Walt) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis humilis E.E. Nel & van der Walt (1968)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (3.0–6.0×(5.0–8.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is off-white, smooth, glistening, and butyrous with an entire edge.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–/l
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	–
5-Keto-D-gluconate	–	Ethylamine	–
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	–	Growth at 35°C	w
Creatinine	–	Growth at 370°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 47.1, CBS 5658 (T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 5658, Bantu beer.

Type strain: CBS 5658.

Comments: Kurtzman and Robnett (unpublished data) found *C. humilis* and *C. milleri* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.63. *Candida incommunis* Y. Ohara, Nonomura & T. Yamazaki (1965)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are elongate, cylindrical, (1.5–2.5)×(6.0–12.0) μm, single and in chains (Fig. 232).

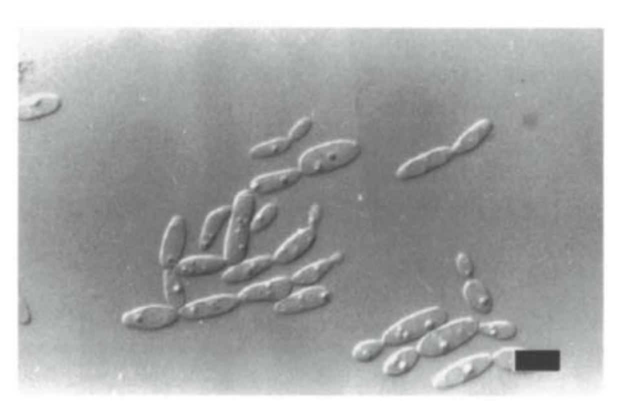


Fig. 232. *C. incommunis*, CBS 5604. After 5 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, long pseudohyphae of branched chains of cylindrical cells are present. Aerobic growth is off-white with a wrinkled center, a smoother periphery and some pseudomycelial development at the margin.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	s/–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–/l	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+/l	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–/l	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	–
D-Glucuronate	+	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	+	Pyridoxine-free	+
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	+	Growth at 35°C	+
Cadaverine	+	Growth at 37°C	w/–
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 8 (Yamada and Kondo 1972a).

Mol% G+C: 48.9, CBS 5604 (T_m: Meyer et al. 1984); 44.1, AJ 5007 (T_m: Nakase and Komagata 1971f).

Origin of the strain studied: CBS 5604, grape must, Japan.

Type strain: CBS 5604.

64.64. *Candida inconspicua* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Torulopsis inconspicua Lodder & Kreger-van Rij (1952)

Torulopsis inconspicua Lodder & Kreger-van Rij var. *filiforme* Dietrichson (1954)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–5.0)×(5.0–11.0) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, primitive pseudohyphae of short chains of ovoidal cells are sometimes present. Aerobic growth is off-white, semidull, soft, mostly smooth and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 8 (Yamada and Kondo 1972a).

Mol% G+C: 36.3, AJ 5139 (T_m: Nakase and Komagata 1971e); 36.7, CBS 180 (BD: Meyer et al. 1984).

Origin of the strains studied: CBS 180, CBS 1735, CBS 2833, sputum; CBS 990, tongue.

Type strain: CBS 180.

64.65. *Candida insectalens* (D.B. Scott, van der Walt & van der Klift) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis insectalens D.B. Scott, van der Walt & van der Klift (van der Walt et al. 1971b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (2.0–3.5)×(2.0–3.5) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudomycelial development is not evident. Aerobic growth is off-white, smooth, and convex with an entire margin.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	l	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	l	Ribitol	l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+/l
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	+/–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	–/l

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	v	Urease	–
Propane 1,2 diol	–	Biotin-free	–/l
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 44.9, CBS 6036 (T_m: Stenderup et al. 1972).

Origin of the strains studied: CBS 6036, CBS 6149, insect tunnels in trees, South Africa.

Type strain: CBS 6036.

64.66. *Candida insectamans* D.B. Scott, van der Walt & van der Klift (van der Walt et al. 1972)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spheroidal, ovoidal to long ovoidal, ellipsoidal, and cylindrical, (1.5–6.5)×(2.5–12.0) μm, single, in pairs and chains (Fig. 233). Pseudohyphae are present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of cylindrical cells with some verticils of ovoidal cells. Aerobic growth is cream-colored with a raised, wrinkled central area, smooth near the periphery and with a fringed margin.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		



Fig. 233. *C. insectamans*, CBS 6033. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–/l
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	l
Melezitose	l	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 35.9, CBS 6033 (BD: Meyer et al. 1984).

Origin of the strain studied: CBS 6033, frass of beetle larvae in trees, South Africa.

Type strain: CBS 6033.

64.67. *Candida insectorum* D.S. Scott, van der Walt & van der Klift (van der Walt et al. 1972)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to cylindrical, (1.5–2.0)×(3.0–8.0) μm, single and in small groups. Pseudohyphae are present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae of branched chains of cylindrical cells and septate hyphae are present. Aerobic growth is yellowish-white to light tan-colored, mostly creamy, but with some wrinkled areas and a fringed border.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	s/–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–/l	D-Mannitol	+
Melibiose	–/l	D-Glucitol	+
Raffinose	l	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+/l	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	v
Nitrite	–	0.1% Cycloheximide	v
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 39.5, CBS 6213 (T_m: Stenderup et al. 1972).

Origin of the strains studied: CBS 6213, CBS 6214, insect tunnels in trees, South Africa.

Type strain: CBS 6213.

64.68. *Candida intermedia* (Ciferri & Ashford) Langeron & Guerra (1938)

Synonyms:

Blastodendrion intermedium Ciferri & Ashford (1929)
Cryptococcus intermedius (Ciferri & Ashford) Nannizzi (1934)
Mycotorula intermedia (Ciferri & Ashford) Krasil'nikov (1954c)
Candida intermedia (Ciferri & Ashford) Langeron & Guerra var. *ethanophila* Verona & Zardetto de Toledo (1954)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.4–4.0) × (3.0–6.0) μm, single and in pairs; elongate cells are also present. A thick, wrinkled pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of many branched chains of cylindrical cells. Aerobic growth is white to cream-colored, smooth to slightly wrinkled and soft.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/s	Raffinose	v
Sucrose	+	Trehalose	s/–
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–/l
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+/l
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+/l
Melezitose	+	Salicin	+
Inulin	–/l	D-Gluconate	–/l
Soluble starch	v	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–/l	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	–/l	Hexadecane	n
L-Rhamnose	v	Nitrate	–
D-Glucosamine	v	Vitamin-free	–/l

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	v
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	v	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	v	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	v
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G + C: 43.7, AJ 4621; 44.4, AJ 4632; 44.4, AJ 4619 (CBS 592) (T_m: Nakase and Komagata 1971f).

Origin of the strains studied: CBS 572, feces; CBS 2044, washed beer bottle; CBS 2049, beer; CBS 2879, soil; CBS 5159, skin; CBS 5310, grape (*C. intermedia* var. *ethanophila*); CBS 5460, fruit.

Type strain: CBS 572.

64.69. *Candida ishiwadae* Sugiyama & S. Goto (1969)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (1.5–5.0) × (3.0–6.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, a few primitive pseudohyphae consisting of chains of elongated cells are present. Aerobic growth is white to slightly cream-colored, mucoid, moist, smooth and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	s/–	Raffinose	–
Sucrose	s/–	Trehalose	s
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–/l	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–/l
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+/l
Melezitose	+/l	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+/l
D-Xylose	+	Succinate	+
L-Arabinose	l	Citrate	+
D-Arabinose	+/l	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	l	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	+
Xylitol	+/l	10% NaCl/5% glucose	+
L-Arabinitol	l	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	v	Biotin-free	v
Butane 2,3 diol	v	Pyridoxine-free	+
Nitrite	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 35°C	+/w
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 36.7, CBS 6022 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 5526, unknown; CBS 6022, soil.

Type strain: CBS 6022.

64.70. *Candida karawaiewii* Yarrow & S.A. Meyer (1978)

Synonym:

Torulopsis karawaiewii Jurzitza (1970) nom. nud.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (2.5–5.0)×(2.5–5.0) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, there is no evidence of pseudohyphae. Aerobic growth is white to cream-colored, smooth, soft, butyrous and entire.

Fermentation:

Glucose	+/s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–/l
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	–
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	n
Butane 2,3 diol	–	Pyridoxine-free	n
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	w
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 8 (Suzuki et al. 1994).

Mol% G+C: 34.4, 35.9, 37.8, 37.3, CBS 5214 (T_m: Stenderup et al. 1972; T_m: Suzuki et al. 1994; BD: Meyer et al. 1984; T_m: Meyer, unpublished data, respectively).

Origin of the strains studied: CBS 2751, CBS 5214, CBS 5215, associated with bark beetles and their larvae (*Ernobius* spp.).

Type strain: CBS 5214.

Comments: Suzuki et al. (1994) described a new species, *C. stellimalicola,* that resembles *C. karawaiewii, C. diversa* and *C. silvae* in physiological and morphological properties. See Comments for *C. diversa* for more information. Kurtzman and Robnett (unpublished data) found *C. ernobii* and *C. karawaiewii* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific. Furthermore, the sequence of *Pichia holstii* differed by only two nucleotides suggesting that it is either the teleomorph or a sibling species.

64.71. *Candida krissii* S. Goto & Iizuka (Goto et al. 1974)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, long oval to cylindrical, (2.0–4.0)×(4.0–7.0) µm, single and in pairs (Fig. 234).

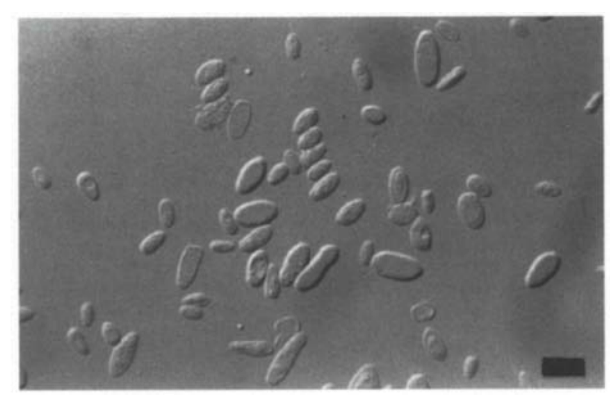


Fig. 234. *C. krissii*, CBS 6519. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, abundant development of pseudohyphae consists of branched chains of cylindrical cells with verticils of ovoidal blastoconidia. Aerobic growth is off-white, smooth, shiny, butyrous and with an entire to undulating margin.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–/l	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	l
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	s
Xylitol	–	10% NaCl/5% glucose	+/l
L-Arabinitol	–	Starch formation	–
Arbutin	v	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 43.4, CBS 6519 (T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 6519, sea water.

Type strain: CBS 6519.

Comments: Kurtzman and Robnett (1997) found *C. krissii* and *C. zeylanoides* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.72. *Candida kruisii* (Kocková-Kratochvílová & Ondrušová) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis kruisii Kocková-Kratochvílová & Ondrušová (1971)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, long-ovoidal to cylindrical, (2.0–4.0) × (5.0–8.0) µm, single, in pairs and chains (Fig. 235).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with ovoidal and elongate blastoconidia in verticils and short chains. Aerobic growth is off-white to cream-colored, smooth, butyrous, shiny and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	l	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	l	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	+



Fig. 235. *C. kruisii*, CBS 6451. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–/s
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Nakase et al. 1988b).

Mol% G+C: 44.6, 45.4, 44.2, CBS 6451 (T_m: Meyer et al. 1984; T_m and HPLC: Nakase et al. 1988b, respectively).

Origin of the strain studied: CBS 6451, fungus, *Boletus purpureus*.

Type strain: CBS 6451.

64.73. *Candida krusei* (Castellani) Berkhout (1923)

See *Issatchenkia orientalis*: p. 222

64.74. *Candida lactis-condensi* (B.W. Hammer) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Torula lactis-condensi B.W. Hammer (1919)
Torulopsis lactis-condensi (B.W. Hammer) Lodder & Kreger-van Rij (1952)
Torulopsis caroliniana Etchells & T.A. Bell (1950b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (1.5–2.5)×(3.0–7.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is white, butyrous, soft and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+/l	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	–
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	n	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–/l
Nitrite	+	0.1% Cycloheximide	–
Cadaverine	–	Growth at 35°C	w/–
Creatinine	–	Growth at 37°C	–
L-Lysine	v		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 8 (Yamada and Kondo 1972a).

Mol% G+C: 42.4, type strain (T_m: Nakase and Komagata 1971e); 43.2, CBS 52 (BD: Meyer et al. 1984).

Origin of the strains studied: CBS 52, sweetened condensed milk; CBS 53, CBS 54 (syntype of *Torulopsis caroliniana*), fermenting brined cucumbers.

Type strain: CBS 52.

Comments: This species usually grows very poorly and is difficult to maintain in culture.

64.75. *Candida laureliae* C. Ramírez & A. González (1984f)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (3.0–4.0)×(5.0–7.0) μm, single and in pairs (Fig. 236).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells, sometimes with verticils of ovoidal blastoconidia. Aerobic growth is white, shiny, and smooth with some tufts of pseudohyphal development.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

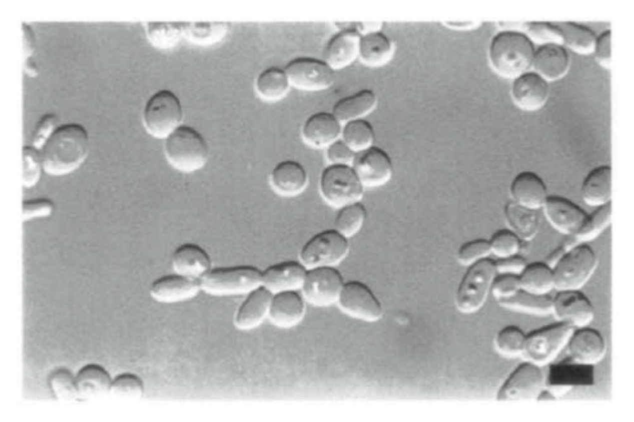


Fig. 236. *C. laureliae*, CBS 8180. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	l
Sucrose	–	Glycerol	l
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–/l
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 44.9, type strain (T_m: Tengku Zainal Mulok 1988).

Origin of the strain studied: CBS 8180, rotten wood, Chile.

Type strain: CBS 8180.

Comments: This species resembles *Pichia pini* in morphology and physiology. Kurtzman and Robnett (1997) found *C. laureliae* and *C. ralunensis* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, and to differ by only one nucleotide from *C. boleticola,* thus indicating the three taxa to be conspecific.

64.76. *Candida lipolytica* (Harrison) Diddens & Lodder (1942)

See *Yarrowia lipolytica*: p. 420

64.77. *Candida llanquihuensis* C. Ramírez & A. González (1984f)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.5–7.0)×(5.0–7.0) μm, single and in pairs (Fig. 237).

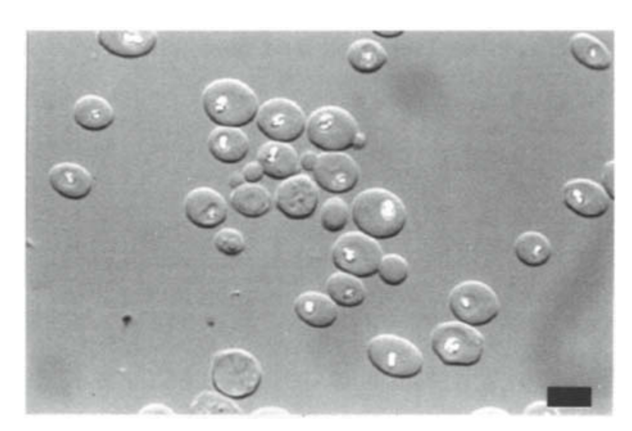


Fig. 237. *C. llanquihuensis*, CBS 8180. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells with verticils of ovoidal blastoconidia. Aerobic growth is white, waxy, and dull with an irregular margin.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	l
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 40.5, type strain (T_m: Tengku Zainal Mulok 1988).

Origin of the strain studied: CBS 8182, rotten wood, Chile.

Type strain: CBS 8182.

64.78. *Candida lusitaniae* van Uden & do Carmo-Sousa (1959)

See *Clavispora lusitaniae*: p. 148

64.79. *Candida lyxosophila* van der Walt, N.P. Ferreira & Steyn (1987a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, ovoidal to ellipsoidal, (3.5–5.0)×(3.5–6.0) µm, single and in pairs. Pseudohyphae are present (Fig. 238).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells with ovoidal blastoconidia; septate hyphae may be present. Aerobic growth is white, smooth, soft and butyrous with a mycelial margin.

Fermentation:

Glucose	s	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	l
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+/l	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+/l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+/l	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	+
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	v
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 37.9, CBS 8194 (van der Walt et al. 1987a); 38.2, CBS 8194 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 7268, CBS 8194, surface woodland soil, South Africa.

Type strain: CBS 8194.

64.80. *Candida magnoliae* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Torulopsis magnoliae Lodder & Kreger-van Rij (1952)
Entelexis magnoliae van der Walt & E. Johannsen (1973)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are uniformly globose to slightly ovoidal, (2.0–4.0)×(3.0–4.5) µm, and occur singly and with buds. A few short chains and small clusters are present (Fig. 239).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is white, smooth, creamy and entire.

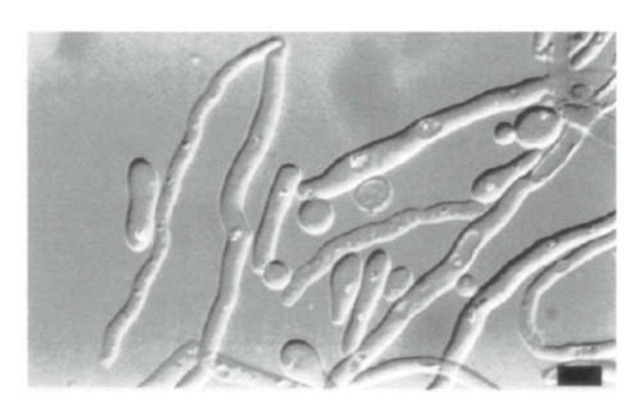


Fig. 238. *C. lyxosophila*, CBS 8194. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

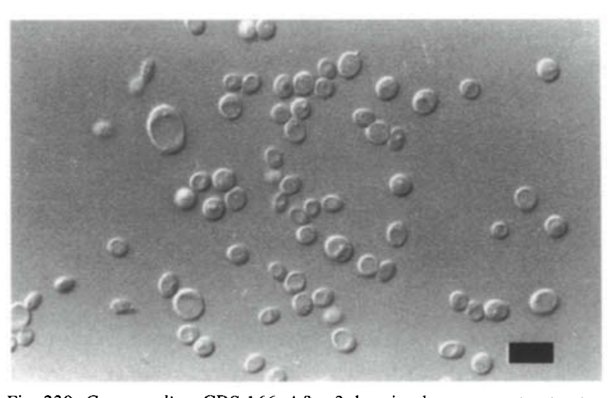


Fig. 239. *C. magnoliae*, CBS 166. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+/l	Ethanol	v
Sucrose	v	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	v	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	v	Biotin-free	–
Propane 1,2 diol	v	Pyridoxine-free	+
Butane 2,3 diol	v	0.01% Cycloheximide	–/l
Nitrite	+	0.1% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 60.0, type strain (T_m: Nakase and Komagata 1971d).

Origin of the strains studied: CBS 166, flower of *Magnolia* sp.; CBS 2250, bumble bee's gut; CBS 2800, gut of a bee, Croatia; CBS 2677, concentrated orange juice, South Africa; CBS 5659, larval feed of *Xylocarpa caffra*; CBS 6396, larval pabulum of *Xylocopa scioensis*, South Africa; CBS 6201, beak of a dead tern; CBS 3086, human sputum, Netherlands; CBS 6424, unknown.

Type strain: CBS 166.

Comments: Nakase et al. (1994a) reported on the heterogeneity among strains of *C. magnoliae*. They used mol% G+C, DNA hybridization and electrophoretic comparisons of seven different enzymes to study 10 strains previously identified as *C. magnoliae*. They found five DNA homology groups and four enzyme groups. One enzyme group was split into two DNA groups. These investigators state that these five DNA groups should be considered distinct species.

64.81. *Candida maltosa* Komagata, Nakase & Katsuya (1964)

Synonyms:

Candida cloacae Komagata, Nakase & Katsuya (1964)

Candida novellus Watanabe, Shimada, Kawaharada, K. Suzuki & Tanaka (1973) nom. nud.

Candida subtropicalis Nakase, Fukazawa & Tsuchiya (1972)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.0–5.0)×(4.0–7.0) μm, and occur singly or in pairs or small groups (Fig. 240).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of cylindrical cells with verticils of ovoidal blastoconidia. Aerobic growth is white, creamy, soft, smooth or slightly folded with a fringed margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	–
Sucrose	+	Trehalose	+
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+/l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+/l	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/l
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–/l
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+/l	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+/l
Xylitol	+/l	10% NaCl/5% glucose	v
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–/l	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a; Kaneko et al. 1977).
Mol% G+C: 36.3, AJ 4718 (T_m: Nakase and Komagata 1971f); 35.6–36.8, 6 strains (T_m: Meyer et al. 1975); 36.1, CBS 5611; 36.6, ATCC 20184; 37.3, #36 (CBS 6658) and CBS 6465 (T_m: Kaneko et al. 1977); 36.6, CBS 6658; 36.8, CBS 6680 (T_m: Su and Meyer 1991).

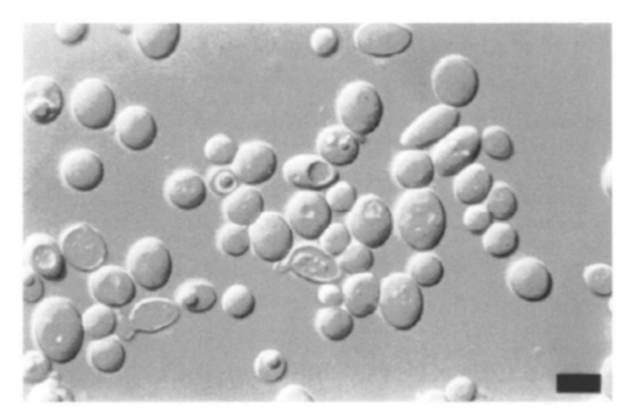


Fig. 240. *C. maltosa,* CBS 6680. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Origin of the strains studied: CBS 5611, neutralizing tanks for monosodium glutamate; CBS 5612 (type strain of *C. cloacae*), mud; CBS 6465 (type strain of *C. subtropicalis*), atmosphere; CBS 6658 (*C. novellus*), CBS 6680, soil, Japan; CBS 7327, waste water, Germany.
Type strain: CBS 5611.

Comments: Meyer et al. (1975) and Kaneko et al. (1977) employed DNA reassociation to demonstrate that *C. maltosa* is distinct from *C. sake* and *C. tropicalis*. These species are separated on the basis of maximum temperature for growth, assimilation of soluble starch and growth in the presence of cycloheximide.

64.82. *Candida maris* (van Uden & Zobell) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis maris van Uden & Zobell (1962)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.0–4.0) × (2.5–4.5) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is cream-colored, soft, smooth and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	+
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+/l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+/l
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+/l	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	w
L-Lysine	+	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.
Co-Q: 7 (Yamada and Kondo 1972a).
Mol% G+C: 47.3, CBS 5151 (T_m: Nakase and Komagata 1971d); 50.9, CBS 5151 (BD: Meyer et al. 1984).

Origin of the strain studied: CBS 5151, sea water, Torres Strait, Australia.
Type strain: CBS 5151.

64.83. *Candida maritima* (Siepmann) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)

Synonyms:
Trichosporon maritimum Siepmann (Siepmann and Höhnk 1962)
Candida maritima (Siepman) van Uden & H.R. Buckley (1970) nom. inval.
Candida pilmaiquensis C. Ramírez & A. González (1984g)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are primarily ovoidal, (3.0–5.0) × (3.5–8.0) μm, single and in pairs; cylindrical cells also are present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with verticils of ovoidal blastoconidia. Aerobic growth is off-white to cream-colored, soft, smooth to wrinkled, and glistening with an irregular or mycelial border.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	s/–
Sucrose	s/–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–/l	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+/l
D-Xylose	+	Succinate	+
L-Arabinose	–/l	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	v	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	v	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	v
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 45.4, type strain (T_m: Meyer et al. 1984); 46.8, CBS 8176 (T_m: M.Th. Smith, personal communication).

Origin of the strains studied: CBS 1969, CBS 6069, tanning fluid; CBS 4112, tunnel of ambrosia beetle; CBS 4606, beetle; CBS 5107, sea; CBS 8176 (type strain of *C. pilmaiquensis*), rotten wood, Chile.

Type strain: CBS 5107.

64.84. *Candida melibiosica* H.R. Buckley & van Uden (1968)

Synonyms:

Candida parapsilosis (Ashford) Langeron & Talice var. *hokkai* S. Goto & Yokotsuka (1962b)

Torulopsis navarrensis Moriyon & Ramírez (1974)

Candida navarrensis (Moriyon & C. Ramírez) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Torulopsis pampelonensis C. Ramírez & A.T. Martinez (1978)

Candida pampelonensis (C. Ramírez & A.T. Martinez) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.5–4.5)×(2.5–5.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, primitive pseudohyphae of short, branched chains of cells are present. This type of growth gives a bushy, feathery appearance. Aerobic growth is cream-colored, butyrous, soft, lobated and entire. An occasional tuft of pseudohyphal growth is evident.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–/s
Sucrose	–/s	Trehalose	–/s
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+/l
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+/l	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+/l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	v
Xylitol	+	10% NaCl/5% glucose	+/l
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	v
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	w/–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G + C: 54.5–55.5, 3 strains (T_m: Meyer et al. 1984); 55.9, AJ 4611 (T_m: Nakase and Komagata 1971f): 54.1, CBS 5605; 55.1, CBS 6612; 55.6, CBS 6611; 56.8, CBS 5814 (T_m: Daniel 1983).

Origin of the strains studied: CBS 5605 (type strain of *C. parapsilosis* var. *hokkai*), wine; CBS 5814, soil; CBS 6211, insect tunnels in wood; CBS 6611 (type strain of *Torulopsis pampelonensis*), CBS 6612, (type strain of *Torulopsis navarrensis*), soil, Spain.

Type strain: CBS 5814.

Comments: Daniel (1983) employed DNA reassociation and protein electrophoretic techniques to demonstrate the conspecificity of *Torulopsis pampelonensis, T. navarrensis, C. parapsilosis* var. *hokkai* and *C. melibiosica*.

64.85. *Candida membranifaciens*[1] (Lodder & Kreger-van Rij) Wickerham & K.A. Burton (1954b)

Synonyms:
Candida melibiosi Lodder & Kreger-van Rij var. *membranifaciens* (as *membranaefaciens*) Lodder & Kreger-van Rij (1952)
Candida majoricensis Genestar Serra (1956)
Procandida majoricensis (Genestar Serra) Novák & Zsolt (1961)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.5–6.0)×(3.5–8.0) µm, single and in clusters.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical or long-ovoidal cells with verticils of ovoidal blastoconidia. Septate hyphae may be present. Aerobic growth is off-white to chalky, dull, powdery, dry, and partly wrinkled with a mycelial border.

Fermentation:

Glucose	s	Lactose	–
Galactose	–/s	Raffinose	s
Sucrose	s	Trehalose	s
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+/l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	–/l	DL-Lactate	+/l
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	l
L-Rhamnose	v	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	v
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	v	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–
Nitrite	–	Growth at 35°C	v
Cadaverine	+	Growth at 37°C	v
Creatinine	–	Growth at 40°C	–
L-Lysine	v		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 33.4, CBS 2875 (T_m: Stenderup et al. 1972).

Origin of the strains studied: CBS 1952, urine; CBS 2090, scale insect (*Pulvinaria* sp.), received as *Oospora srinivasii*; CBS 2296, leprous ulcer; CBS 2297, tree (*Eucalyptus saligna*); CBS 2875 (type strain of *C. majoricensis*), tanning fluid; CBS 4430, CBS 4432, unknown; CBS 6060, Lake Champlain, USA.

Type strain: CBS 1952.

64.86. *Candida mesenterica* (Geiger) Diddens & Lodder (1942)

Synonyms:
Pseudomonilia mesenterica Geiger (1910)
Azymoprocandida mesenterica (Geiger) Novák & Zsolt (1961)
Pseudomonilia albomarginata Geiger (1910)
Candida albomarginata (Geiger) Windisch (1953)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, elongate and cylindrical, (1.5–2.0)×(6.0–12.0) µm, in chains and clusters. Pseudohyphal growth is also present. Islets are present or an incomplete pellicle may form.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells. Septate hyphae are present. Aerobic growth is white to cream-colored, dull, wrinkled with a fringed margin.

Fermentation:

Glucose	–/s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–/l	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–/l	Succinate	+
L-Arabinose	–	Citrate	+/l
D-Arabinose	–/l	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

[1] The original spelling, *membranaefaciens*, has been treated as an orthographic error.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	v	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 47.6, one strain (T_m: Meyer and Phaff 1972).

Origin of the strains studied: CBS 602, 2094, beer conduits in breweries; CBS 2095 (type strain of *Pseudomonilia albomarginata*); CBS 6299, beer; CBS 2210, wort.

Type strain: CBS 602.

64.87. *Candida methanosorbosa* (Abe & Yokote) J.A. Barnett, R.W. Payne & Yarrow (1983)

Synonyms:

Torulopsis methanosorbosa Abe & Yokote (Yokote et al. 1974)
Torulopsis nagoyaensis Asai & Makiguchi (Asai et al. 1976)
Candida nagoyaensis (Asai & Makiguchi) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (3.0–4.0)×(3.0–5.0) μm, single and in pairs (Fig. 241).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is off-white to cream-colored, smooth, soft, glistening and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

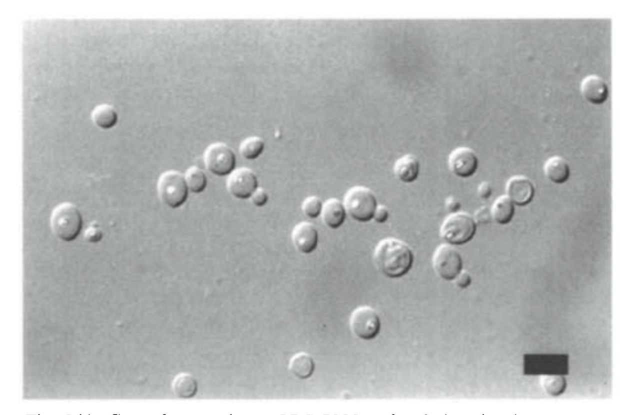


Fig. 241. *C. methanosorbosa*, CBS 7029. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	+
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	+	0.1% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strains studied: CBS 6852 (type strain of *Torulopsis nagoyaensis*); CBS 6853, sewage, Nagoya, Japan; CBS 7029, soil, Japan.

Type strain: CBS 7029.

64.88. *Candida milleri* Yarrow (1978)

Synonyms:

Torulopsis acidi-lactici Nakase, Komagata & Konishi (1977)
Torulopsis holmii (Jörgensen) Lodder var. *acidi-lactici* C. Ramírez & Sierra (1956)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.5–6.0)×(4.0–7.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is off-white to cream-colored, smooth, glistening and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	+/s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–/l	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	–
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	v
Nitrite	–	0.1% Cycloheximide	v
Cadaverine	–	Growth at 35°C	–/l
Creatinine	–	Growth at 37°C	–
L-Lysine	–		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 46.1–46.8, 3 strains (Yarrow 1978); 48.0–48.3, 3 strains (T_m: Nakase et al. 1977).

Origin of the strains studied: CBS 2664 (type strain of *Torulopsis acidi-lactici*), from alpechin, Spain; CBS 6897, CBS 8195, sour dough, USA and Finland, respectively.

Type strain: CBS 6897.

Comments: Kurtzman and Robnett (unpublished data) found *C. humilis* and *C. milleri* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.89. *Candida mogii* Vidal-Leiria (1967)

Synonyms:

Torulopsis miso Mogi (1938a) nom. nud.
Torulopsis miso α var. 1 Mogi (1938a) nom. nud.
Torulopsis miso α var. 2 Mogi (1942) nom. nud.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are long-ovoidal, (1.0–1.5) × (3.0–6.0) μm, single, in pairs and short chains. Pseudohyphae may be present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongated cells, sometimes with verticils of blastoconidia. Aerobic growth is off-white, soft, smooth or wrinkled.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	+
Maltose	–/l		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	l
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–/l
Trehalose	+	Galactitol	–/l
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+/l
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–/l	D-Gluconate	+
Soluble starch	–/l	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	l
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G + C: 46.1, 44.3, CBS 2032 (T_m: Nakase and Komagata 1971f; BD: Kurtzman 1990c, respectively).

Origin of the strain studied: CBS 2032, miso.

Type strain: CBS 2032.

Comments: Kurtzman (1990c) employed DNA reassociation studies to demonstrate that the proposed anamorphic/teleomorphic relationship (Yarrow 1984d) between *C. mogi* and *Zygosaccharomyces rouxii* is not justified. No significant DNA relatedness between the *C. mogii* type strain and any of the nine species included in *Zygosaccharomyces* was revealed.

64.90. *Candida montana* S. Goto & Oguri (1983)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–3.0) × (4.0–5.5) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, short, primitive pseudohyphae are present. Aerobic growth is off-white to cream-colored, butyrous, soft and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (Goto and Oguri 1983).

Mol% G+C: 35.6, CBS 8057 (T_m: Goto and Oguri 1983).

Origin of the strain studied: CBS 8057, wild grapes (*Vitis coignetiae*), Japan.

Type strain: CBS 8057.

Comments: The results here differ from the original description. Goto and Oguri (1983) reported weak fermentation of glucose, very weak assimilation of raffinose, melezitose and α-methyl-D-glucoside, assimilation of glucitol and lactate, growth at 37°C and weak growth in vitamin-free medium.

64.91. *Candida multigemmis* (Buhagiar) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis multigemmis (as *T. multis-gemmis*) Buhagiar (1975)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.5–6.5)×(3.0–6.5) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent or consist of short chains of budding cells. Aerobic growth is white, smooth, creamy, semi-glossy and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–/s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+/l	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	–/l	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	v
Xylitol	+	10% NaCl/5% glucose	v
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	v
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 35.4, type strain (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 6524, CBS 6525, CBS 8140, raspberries, UK.

Type strain: CBS 6524.

64.92. *Candida musae* (Nakase) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis musae Nakase (1971b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.0–5.0)×(4.0–8.0) μm, single and in small groups (Fig. 242).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present, but chains of budding cells are sometimes present. Aerobic growth is white, creamy, smooth, soft and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	l
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	n
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	s	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 47.8, 48.0, CBS 6381 (T_m: Nakase 1971b; T_m: Meyer, unpublished data, respectively).

Origin of the strain studied: CBS 6381, banana, Japan.

Type strain: CBS 6381.

Comments: Kurtzman and Robnett (1997) found *C. fructus* and *C. musae* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

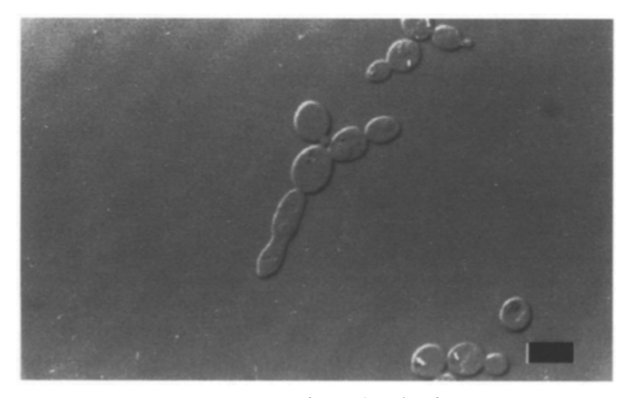


Fig. 242. *C. musae*, CBS 6381. After 5 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

64.93. *Candida naeodendra* van der Walt, E. Johannsen & Nakase (1973)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.0–3.0)×(2.0–5.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are essentially absent, however, short chains of cells are sometimes present. Aerobic growth is cream-colored, soft, smooth with an entire or irregular border.

Fermentation:

Glucose	+	Lactose	–
Galactose	l	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	l	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 38.3, CBS 6032 (T_m: van der Walt et al. 1973).

Origin of the strain studied: CBS 6032, frass of buprestid larvae, South Africa.

Type strain: CBS 6032.

Comments: Kurtzman and Robnett (1997) found *C. diddensiae* and *C. naeodendra* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.94. *Candida nanaspora* Saëz & Rodrigues de Miranda (1988)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.0–3.5)×(3.0–5.0) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is white, butyrous, smooth, soft and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	+
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–/l	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (C.-F. Lee et al. 1994b).

Mol% G+C: 36.6, type strain (T_m: Meyer, unpublished data).

Origin of the strain studied: CBS 7200, male ape (*Cebus apella*), France.

Type strain: CBS 7200.

64.95. *Candida natalensis* van der Walt & Tscheuschner (1957a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to elongate, (2.5–7.0)×(6.0–14.0) µm, in pairs and chains.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells. Aerobic growth is white, soft, and smooth with an irregular edge.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–/s	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	+
D-Xylose	+/l	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	n
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	l
Cadaverine	+	0.1% Cycloheximide	–
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 37.4, CBS 2935 (T_m: Suzuki and Nakase 1988b); 36.8, AJ 4772 (CCY-29-44-1) (T_m: Nakase and Komagata 1971f).

Origin of the strain studied: CBS 2935, soil, South Africa.

Type strain: CBS 2935.

Comments: This species was considered a synonym of *C. sake* (Meyer et al. 1984), but was restored to species status by Suzuki and Nakase (1988b) as the result of DNA hybridization experiments. Also, Fukazawa et al. (1975) showed that *C. natalensis* differed from *C. sake* on the basis of serological analysis and proton magnetic resonance spectra of cell wall mannans, and Montrocher and Claisse (1984) reported that these species differed in antigenic structure as well as the absorption spectra of cytochromes. A new species, *C. fragi*, described by Suzuki et al. (1991) has physiological properties similar to *C. natalensis*, *C. oleophila* and *C. sake*, but is distinguished from these species on the basis of DNA relatedness, electrophoretic enzyme patterns and

proton magnetic resonance (PMR) spectra of the cell wall mannans.

64.96. *Candida nemodendra* (van der Walt, van der Klift & D.B. Scott) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis nemodendra van der Walt, van der Klift & D.B. Scott (1971b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (2.2–4.3) µm, and occur singly and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is off-white, butyrous, smooth, soft, shiny and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	+
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+/l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–/l
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+/l	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–/l

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	+	Biotin-free	–/l
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	v
Cadaverine	+	Growth at 35°C	w
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (J.-D. Lee and Komagata 1980a).

Mol% G+C: 43.2, CBS 6280 (T_m: Stenderup et al. 1972); 39.5, one strain (T_m: J.-D. Lee and Komagata 1980a); 42.4, CBS 6280 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 6185, CBS 6280, tunnels of beetles, South Africa.

Type strain: CBS 6280.

64.97. *Candida nitratophila* (Shifrine & Phaff) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis nitratophila Shifrine & Phaff (1956)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–3.0)×(3.0–6.0) µm, single and in pairs (Fig. 243).

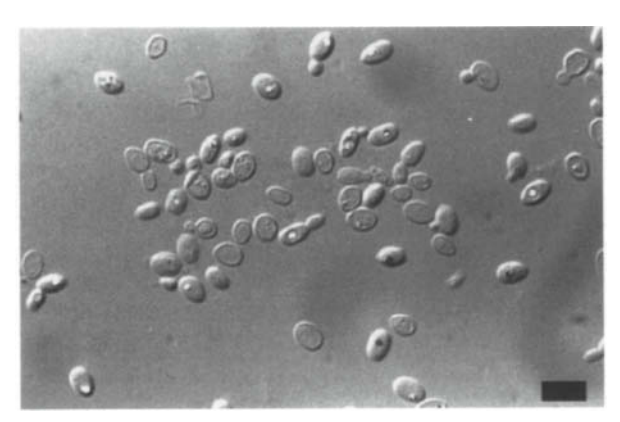


Fig. 243. *C. nitratophila*, CBS 2027. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is off-white, butyrous, mostly smooth, soft, glossy and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	s/–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	+	Methanol	+
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–/l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	l
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 35°C	+/w
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (Yamada and Kondo 1972a).

Mol% G+C: 36.6, CBS 2027 (T_m: Nakase and Komagata 1971b).

Origin of the strain studied: CBS 2027, bark beetles in a conifer.

Type strain: CBS 2027.

64.98. *Candida norvegica* (Reiersöl) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Torulopsis norvegica Reiersöl (1958)
Paratorulopsis norvegica (Reiersöl) Novák & Zsolt (1961)
Torulopsis uvae (Pollacci & Nannizzi) Lodder var. *miso* Mogi (1939)
Torulopsis vanzylii van der Walt & van Kerken (1961b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (3.0–6.0) µm, single and in pairs (Fig. 244).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, short, branched chains of cells are sometimes present. Aerobic growth is white, butyrous, smooth, shiny and entire.

Fermentation:

Glucose	+/s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

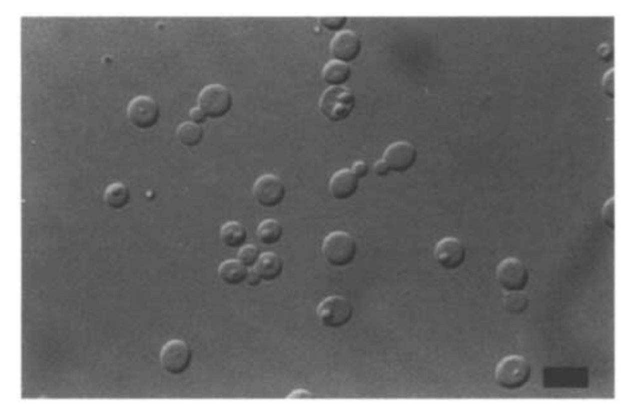


Fig. 244. *C. norvegica*, CBS 4239. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+/l
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–/l
Soluble starch	–	DL-Lactate	+
D-Xylose	+/l	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	v	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–/l
Propane 1,2 diol	+	Pyridoxine-free	–
Butane 2,3 diol	+	0.01% Cycloheximide	v
Nitrite	+	0.1% Cycloheximide	v
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 41.7, CBS 4239 (BD: Stenderup et al. 1972); 40.7, CBS 4239 (BD: Meyer et al. 1984); 41.2, CBS 4239 (Tengku Zainal Mulok 1988).

Origin of the strains studied: CBS 2669, bottle-washing machine; CBS 2670, washed bottles; CBS 2874, miso (*Torulopsis uvae* var. *miso*); CBS 4027, CBS 4239, sputum; CBS 6575, seawater.

Type strain: CBS 4239.

Comments: Tengku Zainal Mulok (1988) showed by DNA reassociation studies that CBS 1784, a strain from apple must that had been considered a representative of *C. norvegica,* had insignificant DNA relatedness with the type strain of the species. Other strains are under investigation to determine their species identity.

64.99. *Candida odintsovae*[2] Bab'eva, Reshetova, Blagodatskaya & Galimova (1989)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, long ovoidal to cylindroidal, (2.0–4.5)×(2.5–11.0) µm, single and with buds. Some cells are slightly curved while others appear claviform or somewhat triangular or tear-drop shaped.

[2] The original spelling, *Candida odintsovii*, has been treated as an orthographic error.

Growth in yeast nitrogen base + 0.5% glucose: After 3 days at 25°C, a thick, folded pellicle is present and growth adheres to the walls of the tube.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, abundant, entwined pseudohyphal growth is present. Some cells are curved and some of the growth appears 'bushy' or feathery and does not extend far from the streak. Aerobic growth is white to off-white, smooth, and dull to somewhat chalky with a mycelial margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–/l	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	l	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (Bab'eva et al. 1989).

Mol% G + C: 36.0–36.6, 5 strains (HPLC: Bab'eva et al. 1989); 38.3, CBS 6025 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 6025, CBS 6026, sap of birch (*Betula verrucosa*), Russia.

Type strain: CBS 6026.

Comments: Bab'eva et al. (1989) reported the fermentation of maltose (weak, slow), the assimilation of 5-ketogluconate and the maximum growth temperature of 42°C. They pointed out that the most characteristic feature of this species is its high glucosidase activity.

64.100. *Candida oleophila* Montrocher (1967)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, a few cells are spheroidal, but most are ovoidal or long-ovoidal to elongate, (2.9–4.3) × (4.3–6.5) μm. They occur singly, in pairs, and in short branched and unbranched chains. Slender cylindrical cells, (1.5–3.6) × (4.3–9.4) μm, are also present, as well as cells that are slightly curved. A thin, delicately wrinkled pellicle is present (Fig. 245).

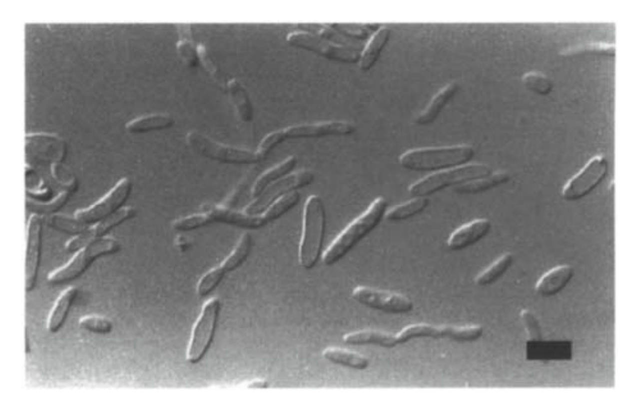


Fig. 245. *C. oleophila*, CBS 2219. After 5 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells, sometimes with verticils of ovoidal blastoconidia. Aerobic growth is off-white, dull, both smooth and delicately wrinkled, and the margin is usually entire with a few tufts of pseudohyphal growth.

Fermentation:

Glucose	+	Lactose	–
Galactose	+/s	Raffinose	–
Sucrose	–/l	Trehalose	–/l
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+/l
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	–/l	Hexadecane	v
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+/l	10% NaCl/5% glucose	v
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Nakase et al. 1988b).

Mol% G+C: 40.7, CBS 2219; 41.5, CBS 4371 and CBS 6106 (T_m: Meyer et al. 1984); 42.2, NRRL Y-2317 (CBS 2219) (T_m: Nakase and Komagata 1971f); 43.7, UCD-FST 62-4=CBS 2219 (T_m: Meyer and Phaff 1972); 41.7, 40.1, CBS 2219 (T_m and HPCL, respectively: Nakase et al. 1988b).

Origin of the strains studied: CBS 2219, olives, Italy; CBS 2220, a flower; CBS 4371, cider, UK; CBS 6106, tonic water.

Type strain: CBS 2219.

Comments: The three strains identified as *C. oleophila* were at one time included in *C. sake*. Meyer and Simione (1978; unpublished data) showed by DNA reassociation that these strains were *C. oleophila*. Fukazawa et al. (1975) demonstrated that *C. oleophila* and *C. sake* differed on the basis of antigenic structure and the proton magnetic resonance spectra of their cell wall mannans. Also, Montrocher (1980) demonstrated that these species are serologically distinct and Montrocher and Claisse (1984) showed that they have different cytochrome absorption spectra as well as antigenic differences. Three species, *C. rignihuensis, C. railenensis* and *Apiotrichum osvaldi,* described by Ramírez and González (1984c,g), were thought to be members of *C. oleophila* (Barnett et al. 1990). Tengku Zainal Mulok (1988) showed by DNA reassociation that the latter two species were not representatives of *C. oleophila,* but they themselves are conspecific. *C. railenensis* has priority. *C. rignihuensis* and several strains in the CBS collection considered to be members of *C. oleophila* based on morphological and physiological properties demonstrated different whole cell protein electrophoretic patterns and different nuclear DNA restriction fragment length polymorphisms (Park and Meyer, unpublished data). These strains have been removed from *C. oleophila* for further study. *C. fragi,* a new species described by Suzuki et al. (1991) has physiological properties similar to *C. oleophila, C. sake* and *C. natalensis,* but it is distinct from them on the basis of DNA relatedness, electrophoretic enzyme patterns and proton magnetic resonance (PMR) spectra of the cell wall mannans. These investigators noted that *C. fragi* differs from *C. oleophila* by its inability to utilize trehalose, to ferment galactose and to grow in the presence of 100 ppm cycloheximide.

64.101. *Candida oregonensis* Phaff & do Carmo-Sousa (1962)

Synonym:

Candida obtusa Dietrichson ex van Uden & H.R. Buckley var. *oregonensis* (Phaff & do Carmo-Sousa) Montrocher (1967)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–3.5)×(2.5–5.0) µm, single and in chains.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells, often with dense verticils of ovoidal blastoconidia. Aerobic growth is white to cream-colored, butyrous, smooth, glistening and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	–/l	Hexadecane	l
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	v	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–/l
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 48.1, 48.0, type strain (T_m: Meyer and Phaff 1972; T_m: Nakase and Komagata 1971f, respectively).

Origin of the strains studied: CBS 5036, insect frass; CBS 5623, alpechin.

Type strain: CBS 5036.

64.102. *Candida ovalis* Kumamoto & Yamamoto (Kumamoto et al. 1986)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (2.5–4.5)×(3.0–4.5) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is white to cream-colored, butyrous, moist, soft and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	+
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (Kumamoto et al. 1986).

Mol% G+C: 35.8, CBS 7298 (T_m: Kumamoto et al. 1986).

Origin of the strain studied: CBS 7298, soil, Japan.

Type strain: CBS 7298.

64.103. *Candida palmioleophila* Nakase & M. Itoh (Nakase et al. 1988a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are primarily globose with some that are slightly ovoidal, (1.5–4.0)×(1.5–5.0) µm, and occur singly, in pairs, short chains and small clusters. A thin pellicle is present which eventually drops to the bottom of the tube (Fig. 246).

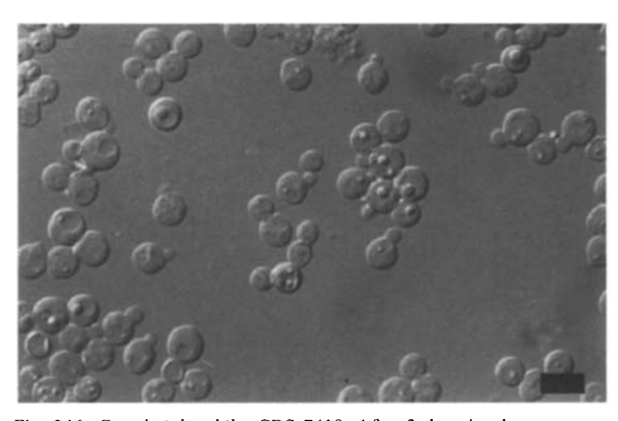


Fig. 246. *C. palmioleophila*, CBS 7418. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, primitive pseudohyphae consisting of short, branched chains of ovoidal cells are present. Aerobic growth is white, butyrous, smooth, soft and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	l	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	+	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Nakase et al. 1988a).
Mol% G+C: 39.4, 39.7, CBS 7418 (T_m and HPLC: Nakase et al. 1988a, respectively).

Origin of strain studied: CBS 7418, soil, Japan.
Type strain: CBS 7418.

Comments: Nakase et al. (1988a) pointed out that *C. palmioleophila* has characteristics similar to *C. famata* and *C. saitoana,* but can be differentiated from them on the basis of its high maximum growth temperature. Furthermore, DNA reassociation studies support the separation of these species. Interestingly, these investigators found 40.3% DNA relatedness between *C. palmioleophila* and *C. parapsilosis,* but also revealed that *C. palmioleophila* lacks antigen 13 which is specific for antigenic Group IV of which *C. parapsilosis* is a member.

64.104. *Candida paludigena* Golubev & Blagodatskaya (Golubev et al. 1981)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–3.5)×(4.0–7.0) µm, single and in pairs (Fig. 247). Pseudohyphae may be present.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, well-developed pseudohyphae and septate hyphae are present. Aerobic growth is white, butyrous, glistening, and smooth with a fringed border.

Fermentation:

Glucose	s/–	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	–/l
Melibiose	–	D-Glucitol	–
Raffinose	–/l	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 35°C	w
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.
Mol% G+C: 41.5, CBS 8005 (T_m: Meyer, unpublished data).

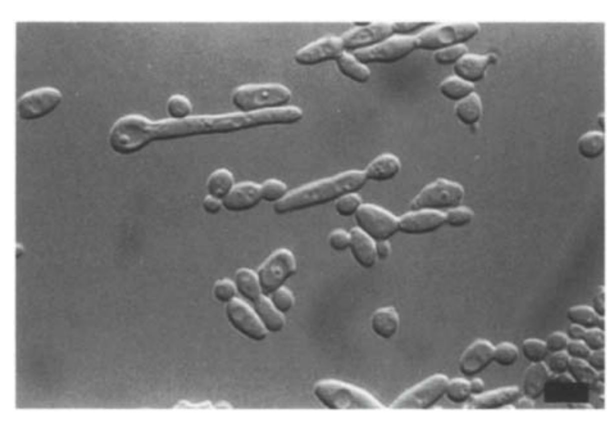


Fig. 247. *C. paludigena,* CBS 8005. After 5 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Origin of the strain studied: CBS 8005, high-moor peat near Moscow.
Type strain: CBS 8005.

Comments: Kurtzman and Robnett (1997) found *C. paludigena* and *C. castrensis* to differ by only two nucleotides in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.105. *Candida parapsilosis* (Ashford) Langeron & Talice (1932)

Synonyms:
Monilia onychophila Pollacci & Nannizzi (Marengo 1926)
Monilia parapsilosis Ashford (1928)
Mycocandida parapsilosis (Ashford) Dodge (1935)
Mycotorula vesica Harrison (1928)
Blastodendrion globosum Zach (Wolfram and Zach 1934a)
Schizoblastosporion globosum (Zach) Dodge (1935)
Blastodendrion gracile Zach (Wolfram and Zach 1934a)
Schizoblastosporion gracile (Zach) Dodge (1935)
Blastodendrion intestinale Mattlet var. *epidermicum* Ciferri & Alfonseca (1931)
Castellania epidermica (Ciferri & Alfonseca) Dodge (1935)
Mycotoruloides unguis (Emile-Weil & Gaudin) Langeron & Talice (1932)

Saccharomyces vercitillatus Dietrichson (1954)
Saccharomyces vossii Dietrichson (1954)
Zymopichia vossii (Dietrichson) Novák & Zsolt (1961)
Candida parapsilosis (Ashford) Langeron & Talice var. *intermedia* van Rij & Verona (1949)
Brettanomyces petrophilum Takeda, Iguchi, Tsuzuki & Nakano (1972)
Torulopsis larvae Kawano, Kojima, Obosawa & Morinaga (1976) nom. inval.
Candida osornensis C. Ramírez & A. González (1984f)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (3.0–4.0)×(5.0–8.0) µm, single and in pairs (Fig. 248). Cylindrical cells up to 20 µm long, as well as pseudohyphae, may also be present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with clusters or chains of blastoconidia. Aerobic growth is white, butyrous, soft, smooth and entire.

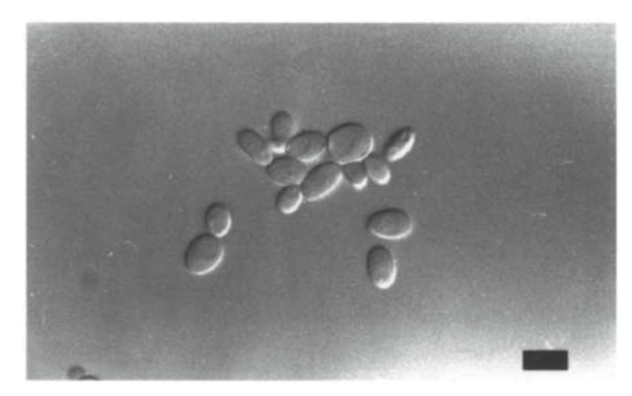


Fig. 248. *C. parapsilosis*, CBS 604. After 5 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	–
Sucrose	–/s	Trehalose	–/s
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+/l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+/l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	+/l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	–/l	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	+/l	Starch formation	–
L-Arabinitol	–/l	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–/l	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	v
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	v
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G + C: 40.0, CBS 604 (T_m: Meyer and Phaff 1969); 40.5, CBS 604 (T_m: Meyer and Phaff 1972); 40.8, CBS 604 (T_m: Stenderup and Bak 1968); 40.5, CBS 6318 (T_m: Meyer, unpublished data); 39.3–40.0, 6 strains (T_m: Nakase and Komagata 1971f); 40.5–41.7, 8 strains (T_m: Su and Meyer 1991).

Origin of the strains studied: CBS 604, sprue, Puerto Rico; CBS 1954, olives, Italy; CBS 2194, nail (*Blastodendrion globosum*); CBS 2196, skin (*Blastodendrion intestinale* var. *epidermicum*); CBS 2197, bladder (*Mycotorula vesica*); CBS 2211, toe; CBS 2215, CBS 2216, pickled cucumbers; CBS 2315, nail (*Monilia onychophila*); CBS 2915 (*Saccharomyces vossii*); CBS 2916, patient (*Saccharomyces verticillatus*), Norway; CBS 6318, CBS 8050, unknown; CBS 8181 (type of *C. osornensis*), rotten tree trunk, Chile.

Type strain: CBS 604.

Comments: *Candida osornensis* demonstrated 100% DNA reassociation with the type strain of *C. parapsilosis* (B. Roy and S.A. Meyer, unpublished data). Lin et al. (1995) showed that strains identified as *C. parapsilosis* based on physiological and morphological characteristics fell into three groups. These groups were defined on the basis of electrophoretic profiles of several enzymes and nucleotide sequences of the internally transcribed spacer, ITS1, next to the 5.8S RNA gene. Two groups have been confirmed by DNA reassociation studies (B. Roy and Meyer, unpublished data). In earlier studies, Meyer and Phaff (1972) showed the lack of DNA relatedness between the type strains of *C. parapsilosis* and *Lodderomyces elongisporus* did not support the proposed anamorphic/teleomorphic relationship of these species. Then, Nakase et al. (1979) demonstrated two different forms of *C. parapsilosis* with similar physiological and serological properties and nuclear magnetic resonance profiles of cell wall mannans. Strains designated *C. parapsilosis* Form I were L-arabinose positive and included the type strain, whereas strains designated *C. parapsilosis* Form II were L-arabinose negative and considered imperfect forms of *L. elongisporus*. Later, Yamazaki and

Komagata (1982a) compared *C. parapsilosis* Form I and Form II on the basis of electrophoretic patterns of ten enzymes and supported the conclusions of Nakase and coworkers. Hamajima et al. (1987) employed DNA base composition and DNA reassociation studies to show that the strains designated *C. parapsilosis* Form II were, indeed, anascosporogenous strains of *L. elongisporus*. It is apparent that the criteria presently employed to identify strains of *C. parapsilosis* are inadequate. The molecular studies being conducted in several laboratories around the world will help to define the '*C. parapsilosis* complex' and provide the criteria necessary to identify the species.



Fig. 249. *C. pararugosa*, CBS 1010. After 5 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

64.106. *Candida pararugosa* Nakase, Komagata & Fukazawa (1978)

Synonym:
Cryptococcus aggregatus Anderson (1917)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are cylindrical, (1.5–3.0) × (6.0–23.0) µm, single, in pairs and short chains (Fig. 249). A white, creeping pellicle composed of tiny, spherical islands like styrofoam discs, covers the entire surface. After one month the pellicle is complete, folded, rough and thick.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, abundant pseudohyphae consist of long, branched chains of cylindrical cells. Aerobic growth is white, raised, and wrinkled to rough with a mycelial border.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	l	Citrate	–
D-Arabinose	–/l	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	+	Biotin-free	–/l
Butane 2,3 diol	+	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 48.3, AJ 4645 (CBS 1010) (T_m: Nakase and Komagata 1971f; T_m: Nakase et al. 1978).

Origin of the strains studied: CBS 1010, from feces, received as *Cryptococcus aggregatus*; CBS 5849, gut of a gecko (*Anatolis bartchi*).

Type strain: CBS 1010.

Comments: The results here differ from the original description in the ability to assimilate glycerol, mannitol, D-glucitol, lactate and succinate.

64.107. *Candida peltata* (Yarrow) S.A. Meyer & Ahearn (1983)

Synonyms:
Torulopsis peltata Yarrow (1968)
Selenotila peltata (Yarrow) Yarrow (1969b)
Selenozyma peltata (Yarrow) Yarrow (von Arx et al. 1977)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to lunate, (2.0–3.5) × (3.0–5.0) µm, single and in pairs. A pellicle may be present.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are not present. Aerobic growth is white to cream-colored, butyrous, and smooth with an entire margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	−
L-Sorbose	+	Ethanol	−
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+/l
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+/l
Lactose	−	D-Mannitol	+
Melibiose	−	D-Glucitol	+
Raffinose	−	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	−	D-Gluconate	−
Soluble starch	+/l	DL-Lactate	−
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	−
D-Ribose	+	Hexadecane	−
L-Rhamnose	+	Nitrate	−
D-Glucosamine	l	Vitamin-free	−

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	−	L-Lysine	+
5-Keto-D-gluconate	−	Ethylamine	+
Saccharate	−	D-Glucosamine (N)[1]	−
D-Glucuronate	−	50% Glucose	−
Xylitol	+	10% NaCl/5% glucose	−
L-Arabinitol	+	Starch formation	−
Arbutin	+	Urease	−
Propane 1,2 diol	+	Biotin-free	l
Butane 2,3 diol	+	Pyridoxine-free	−
Nitrite	−	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	−	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strains studied: CBS 5564, non-mucoid variant; CBS 5576, from mastitis milk, UK.

Type strain: CBS 5576.

64.108. *Candida petrohuensis* C. Ramírez & A. González (1984h)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.0–4.5)×(3.0–6.0) μm, single and in pairs (Fig. 250). After one month a ring and a thin pellicle may be present.

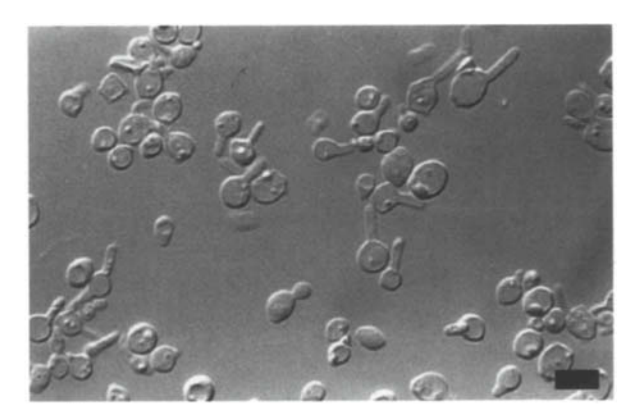


Fig. 250. *C. petrohuensis*, CBS 8173. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, short, dense chains of ovoidal cells are present. Aerobic growth is white to cream-colored, butyrous, smooth, soft and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	−	Methanol	−
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	−
Cellobiose	+	Ribitol	l
Trehalose	+/l	Galactitol	−
Lactose	−	D-Mannitol	l
Melibiose	−	D-Glucitol	+
Raffinose	−	α-Methyl-D-glucoside	l
Melezitose	−	Salicin	−/l
Inulin	−	D-Gluconate	−
Soluble starch	−	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	−/l	Citrate	−
D-Arabinose	−	Inositol	−
D-Ribose	−	Hexadecane	−
L-Rhamnose	−	Nitrate	−
D-Glucosamine	−/l	Vitamin-free	−

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	−	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	−	D-Glucosamine (N)[1]	−
D-Glucuronate	l	50% Glucose	−
Xylitol	−	10% NaCl/5% glucose	−
L-Arabinitol	−	Starch formation	−
Arbutin	l	Urease	−
Propane 1,2 diol	+	Biotin-free	−
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	−	0.1% Cycloheximide	+
Cadaverine	+	Growth at 25°C	+
Creatinine	−	Growth at 30°C	−

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 46.1, CBS 8173 (T_m: Tengku Zainal Mulok 1988).

Origin of the strain studied: CBS 8173, rotten wood, Chile.

Type strain: CBS 8173.

Comments: Kurtzman and Robnett (1997) found *C. petrohuensis, C. ancudensis* and *C. drimydis* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, thus indicating the three taxa to be conspecific.

64.109. *Candida pignaliae* (F.H. Jacob) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis pignaliae F.H. Jacob (1970)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose, (3.5–7.0) μm, single, in pairs and small groups.

Dalmau plate culture on corn meal agar: After

14 days at 25°C, pseudohyphae are not present. Aerobic growth is white to cream-colored, butyrous, smooth, glistening and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+
L-Sorbose	–/l	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	l	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (C.-F. Lee et al. 1994b).

Mol% G+C: 42.7, CBS 6071 (BD: Meyer et al. 1984).

Origin of the strain studied: CBS 6071, tanning fluid, France.

Type strain: CBS 6071.

Comments: The value 43.7 mol% G+C for this species reported by Stenderup et al. (1972) should have been 42.7.

64.110. *Candida pini* (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Torulopsis pinus Lodder & Kreger-van Rij (1952)

Paratorulopsis pinus (Lodder & Kreger-van Rij) Novák & Zsolt (1961)

Growth in glucose-yeast extract-peptone broth: After 3 days at 25°C, the cells are globose, (3.5–5.0)×(3.5–5.0) µm, single, in pairs and small groups (Fig. 251).

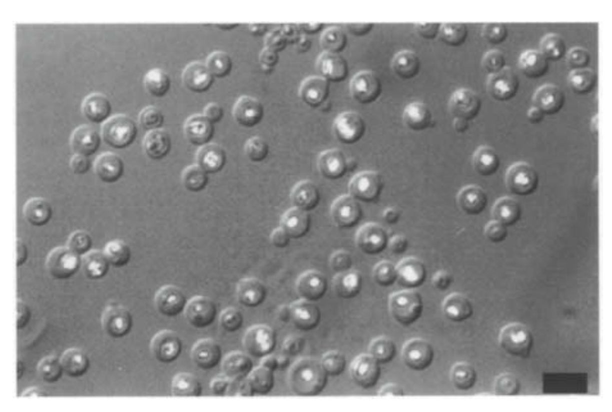


Fig. 251. *C. pini*, CBS 970. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is white to cream-colored, soft, mucoid and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–/s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	l	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (Yamada and Kondo 1972a).

Mol% G+C: 37.3, type strain (T_m: Nakase and Komagata 1971d).

Origin of the strain studied: CBS 970, water-logged heart of a pine tree.

Type strain: CBS 970.

64.111. *Candida populi* Hagler, Mendonça-Hagler & Phaff (1989)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are uniformly globose, (1.0–2.0) μm, and occur singly, budding in short chains and clusters.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. However, there may be some short chains of cells evident. Aerobic growth is white, butyrous, smooth, soft and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	w/s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+/l
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	+/l	Urease	–
Arbutin	+	Biotin-free	n
Propane 1,2 diol	+	Pyridoxine-free	n
Butane 2,3 diol	+	0.1% Cycloheximide	+
Nitrite	+	Growth at 30°C	+
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 8 (Hagler et al. 1989).

Mol% G+C: 37.4–38.9, 6 strains (BD: Hagler et al. 1989).

Origin of the strains studied: CBS 7351, 7352, 7353, exudates of trees (*Populus* and *Betula* species).

Type strain: CBS 7351.

64.112. *Candida pseudointermedia* Nakase, Komagata & Fukazawa (1976)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to short ovoidal, (4.0–5.0)×(4.0–6.0) μm, single and in pairs. A ring and a sediment are present.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with verticils of subglobose to ovoidal blastoconidia. Aerobic growth is white to cream-colored, butyrous, dull, dry, mostly smooth with some wrinkled areas and fringed margins.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	+	Trehalose	+
Maltose	s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	l
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–/l	D-Gluconate	+
Soluble starch	–/l	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	l	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	+	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	l
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 44.9, CBS 6918 (T_m: Nakase et al. 1976).

Origin of the strain studied: CBS 6918, fish paste, Japan.

Type strain: CBS 6918.

64.113. *Candida pseudolambica* M.Th. Smith, Poot & Kull (1989a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are mostly globose or slightly ovoidal, (3.0–5.0)×(3.0–5.0) µm, and occur singly. Some pairs and small clusters are present (Fig. 252).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, primitive pseudohyphae are present. Aerobic growth is white to cream-colored, butyrous, smooth and entire.

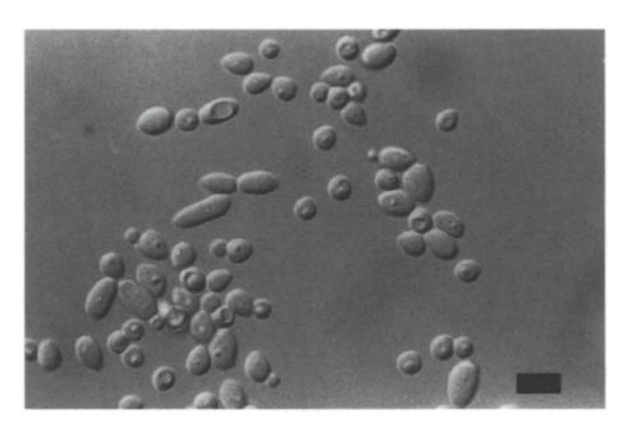


Fig. 252. *C. pseudolambica*, CBS 2063. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+/l	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+/l	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 30.8–31.4, 5 strains (T_m: Smith et al. 1989a).

Origin of the strains studied: CBS 2058, feces; CBS 2063, silage, UK; CBS 2067 (ATCC 4673), CBS 4325 and CBS 4326, soil, Finland.

Type strain: CBS 2063.

Comments: The strains included in this species were formerly identified as *C. lambica*, but were determined to be a new species after comparisons by DNA/DNA hybridization (Smith et al. 1989a). Two other species with similar mol% G+C, *C. ethanolica* (29.3) and *C. rugopelliculosa* (30.0), also showed no significant DNA relatedness with *C. pseudolambica*.

64.114. *Candida psychrophila* (S. Goto, Sugiyama & Iizuka) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis psychrophila S. Goto, Sugiyama & Iizuka (1969)

Growth in glucose–yeast extract–peptone broth: After 3 days at 15°C, the cells are spheroidal, (2.5–4.5) µm, single, in pairs or small clusters.

Dalmau plate culture on corn meal agar: After 14 days at 15°C, pseudohyphae are absent. Aerobic growth is white, butyrous, smooth, soft and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–/l	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	–
5-Keto-D-gluconate	n	Ethylamine	–
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	s
Xylitol	+	10% NaCl/5% glucose	n
L-Arabinitol	+	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	n
Butane 2,3 diol	–	Pyridoxine-free	n
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	–	Growth at 19°C	–
Creatinine	–		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 36.3, 35.9, CBS 5956 (T_m: Nakase and Komagata 1971d; T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 5956, penguin dung, Antarctica.

Type strain: CBS 5956.

Comments: The only isolate of this species does not grow at temperatures above 17°C. The tests here were performed at 15°C.

64.115. *Candida pulcherrima* (Linder) Windisch (1940)

See *Metschnikowia pulcherrima*: p. 264

64.116. *Candida quercitrusa* (van Uden & do Carmo-Sousa) S.A. Meyer & Phaff ex S.A. Meyer & Yarrow (1998)

Synonyms:

Candida parapsilosis (Ashford) Langeron & Talice var. *querci* van Uden & do Carmo-Sousa (1959)

Candida quercitrusa (van Uden & do Carmo-Sousa) S.A. Meyer & Phaff (1972) nom. inval.

Candida quercus (van Uden & do Carmo-Sousa) Montrocher & Claisse (1984) (as *C. querci*) [non *Candida quercuum* Nakase (1971b)]

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–5.0)×(4.5–7.0) μm, single, in pairs and small groups.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells. Aerobic growth is white, butyrous, soft, and smooth to delicately wrinkled.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	–
Sucrose	–/s	Trehalose	–
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	l
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	l
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–/l
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	l	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–/l
Nitrite	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 41.5, CBS 4412 (T_m: Meyer and Phaff 1972); 39.5 (T_m: Nakase and Komagata 1971f); 40.0 (T_m: Su 1990).

Origin of the strain studied: CBS 4412, insect frass on an oak tree.

Type strain: CBS 4412.

Comments: Meyer and Phaff (1972) employed DNA hybridization to show that *C. parapsilosis* var. *querci* was distinct from *C. parapsilosis* and stated that it should be considered a separate species. However, they did not formally name it. Montrocher and Claisse (1984) used cytochrome absorption spectra and serological procedures to compare these two yeasts and came to the same conclusion. They proposed the name *C. quercus* (published as *querci*). However, this is a later homonym of *C. quercuum* Nakase (1971b) and, therefore, invalid under Article 64.2 of the International Code of Botanical Nomenclature. In the previous edition of *The Yeasts, A Taxonomic Study*, the name *C. quercitrusa* was used for this yeast (Meyer et al. 1984). This name was validated by Meyer and Yarrow (1998).

64.117. *Candida quercuum* Nakase (1971b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to elongate, (1.5–2.5)×(5.0–9.0) μm, single and in pairs (Fig. 253).



Fig. 253. *C. quercuum*, CBS 6422. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells with globose to ovoidal blastoconidia. Aerobic growth is white, butyrous, smooth, dull, dry and entire.

Fermentation:

Glucose	w	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	+
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 38.3, CBS 6422 (T_m: Nakase 1971b); 39.5, CBS 6422 (T_m: Meyer, unpublished data).

Origin of the strain studied: CBS 6422, exudate of an oak tree.

Type strain: CBS 6422.

64.118. *Candida railenensis* C. Ramírez & A. González (1984c)

Synonym:
Apiotrichum osvaldi C. Ramírez & A. González (1984d)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spheroidal to ovoidal or cylindrical, (2.5–4.5) × (4.5–7.5) µm, single, in pairs and chains (Fig. 254). Pseudohyphae are present. A thin, waxy pellicle is evident. After 30 days a thick pellicle is present.

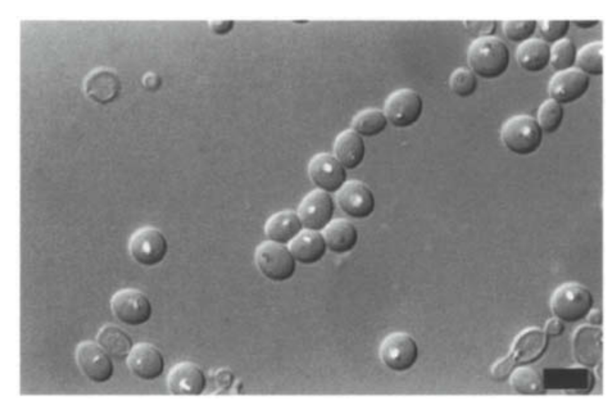


Fig. 254. *C. railenensis*, CBS 8165. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, abundant pseudohyphae with branched chains of cells and ovoidal to elongated blastoconidia are present. True hyphae are also present. Aerobic growth is cream-colored, dull, lacy, and raised with a lobed margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	–	Hexadecane	–/l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+/s
Xylitol	+	10% NaCl/5% glucose	–/l
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	l	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 42.0, CBS 8164; 41.5, CBS 8165 (T_m: Tengku Zainal Mulok 1988).

Origin of the strains studied: CBS 8164, rotten trunk of *Nothofagus dombeyii*; CBS 8165, rotten truck of *Nothofagus obliqua*, Chile.

Type strain: CBS 8164.

Comments: This species and its synonym were considered to be synonyms of *C. oleophila* (Barnett et al. 1990). However, DNA reassociation studies confirmed that they are not members of *C. oleophila*. *C. railenensis* and *Apiotrichum osvaldi* are conspecific and showed no significant DNA relatedness with *C. oleophila*. *C. railenensis* and its synonym also showed no significant DNA relatedness with *C. sake* (Tengku Zainal Mulok 1988).

64.119. *Candida reukaufii* (Grüss) Diddens & Lodder (1942)

See *Metschnikowia reukaufii*: p. 265

64.120. *Candida rhagii* Jurzitza, Kühlwein & Kreger-van Rij (1960)

Synonym:

Candida tropicalis (Castellani) Berkhout var. *rhagii* Diddens & Lodder (1942)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–4.0)×(4.0–6.0) μm, single and in pairs (Fig. 255).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with verticils of ovoidal blastoconidia. Aerobic growth is white and wrinkled with a fringed border.

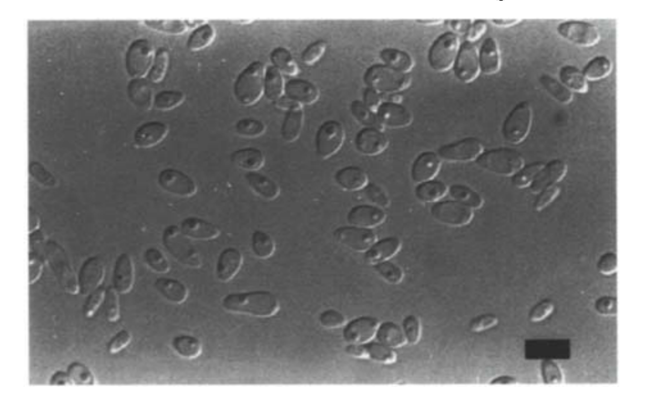


Fig. 255. *C. rhagii*, CBS 4237. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	v
Sucrose	+	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–/l
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	l
L-Rhamnose	v	Nitrate	–
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	v
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+/l	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 42.4, CBS 4237 (T_m: Meyer and Phaff 1972).

Origin of the strains studied: CBS 618, CBS 4237, CBS 4284, from insects and their larvae.

Type strain: CBS 4237.

Comments: Kurtzman and Robnett (1997) found *C. rhagii* and *Pichia heimii* to differ by only two nucleotides in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be either conspecific or sibling species.

64.121. *Candida rugopelliculosa* Nakase (1971a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are mostly ovoidal to elongate, (2.8–5.6)×(5.6–11.2) μm, single, in pairs and short chains. Some spherical cells are present. A wrinkled, folded pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with verticils of blastoconidia. Aerobic growth is white to off-white with raised, folded areas and a mycelial border.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	l
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	l	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 30.0, CBS 6377 (T_m: Nakase 1971a).

Origin of the strain studied: CBS 6377, from soybean protein factory in Japan.

Type strain: CBS 6377.

64.122. *Candida rugosa* (Anderson) Diddens & Lodder (1942)

Synonyms:

Monilia rugosa (Castellani) Castellani & Chalmers (1913)
Mycoderma rugosa Anderson (1917)
Mycotorula rugosa (Saito) Harrison (1928)
?*Candida rugosa* (Anderson) Diddens & Lodder var. *elegans* Dietrichson (1954)
?*Azymocandida rugosa* (Anderson) Novák & Zsolt (1961)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are elongate, (1.5–2.5)×(5.0–11.0) µm, single and in pairs. Islets or a pellicle are present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of densely branched chains of elongate cells. Aerobic growth is off-white, wrinkled and fringed with mycelium.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–/l
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+/l
Melibiose	–	D-Glucitol	+/l
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	+/l
D-Xylose	v	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	l
Xylitol	–/l	10% NaCl/5% glucose	n
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	+/l	Biotin-free	v
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	v

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 50.2–50.7, 4 strains (T_m: Nakase et al. 1978).

Origin of the strains studied: CBS 613, feces of man; CBS 2016, feces of cow; CBS 2274, unknown, from R. Ciferri; CBS 2275, CBS 2277, rancid butter; CBS 6314, unknown, from J.P. van der Walt; CBS 7138, soil in the Netherlands.

Type strain: CBS 613.

64.123. *Candida saitoana* Nakase & M. Suzuki (1985b)

Synonyms:

Torula candida Saito (1922)
Torulopsis candida (Saito) Lodder (1934)
Cryptococcus candidum (Saito) Skinner (1950)
Torulopsis candida (Saito) Lodder var. *marina* Kawano, Kojima, Ohosawa & Morinaga (1976)

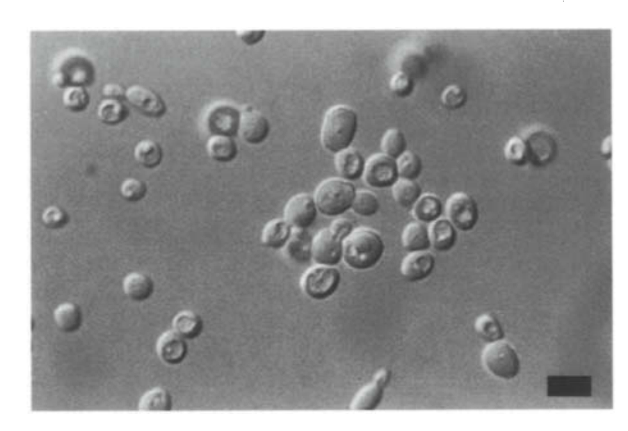


Fig. 256. *C. saitoana*, CBS 940. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (5.0–8.0) × (5.0–10.0) μm, single and in pairs (Fig. 256).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are either absent or primitive pseudohyphae are present. Aerobic growth is off-white, smooth, shiny and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	w
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	v
D-Glucuronate	–	10% NaCl/5% glucose	l
Xylitol	+	Starch formation	–
L-Arabinitol	v	Urease	–
Arbutin	+	Biotin-free	v
Propane 1,2 diol	+	Pyridoxine-free	+
Butane 2,3 diol	+	0.01% Cycloheximide	+
Nitrite	v	0.1% Cycloheximide	–/l
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	v
L-Lysine	+	Growth at 40°C	v

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (T_m: Nakase and Suzuki 1985b).

Mol% G + C: 36.3–37.5, 6 strains (Nakase and Suzuki 1985b).

Origin of strains studied: CBS 940, atmosphere, Japan; CBS 8046, fish food.

Type strain: CBS 940.

Comments: This species is similar to *C. famata*, but differs from it by growing on propane 1,2 diol. Nakase and Suzuki (1985b) distinguished *C. saitoana* from *C. famata* and *C. naganishii* (teleomorph of *Debaryomyces nepalensis*) by proton magnetic resonance spectra of mannans, serological characterization and electrophoretic mobilities of glucose-6-phosphodehydrogenase. As pointed out by these authors, the name *Torula candida* Saito 1922 has priority. However, it cannot be used because the combination *Candida candida* is a tautonym. Therefore, they chose the name *Candida saitoana*, in honor of Dr. Saito who described *Torula candida*. Suzuki and Nakase (1993) described a new species, *C. pseudoglaebosa*, which resembles *C. saitoana* and *C. glaebosa*. See comments for *C. glaebosa*.

64.124. *Candida sake* (Saito & Oda) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)

Synonyms:

Eutorulopsis sake Saito & Oda (1934)
Torulopsis sake (Saito & Oda) Lodder & Kreger-van Rij (1952)
Candida sake (Saito & Oda) van Uden & H.R. Buckley (1970) nom. inval.
Torula lambica Kufferath (1925)
Mycotorula lambica Harrison (1928)
Candida tropicalis (Castellani) Berkhout var. *lambica* (Harrison) Diddens & Lodder (1942)
Candida vanriji Capriotti (1958f)
Candida salmonicola Komagata & Nakase (1965)
Candida australis S. Goto, Sugiyama & Iizuka (1969)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are spheroidal, ovoidal, elongate or cylindrical, (2.5–5.0) × (3.0–6.0) μm, and occur singly, in pairs, short chains and small clusters (Fig. 257). Pseudohyphae may be present. A ring, islets and a thin pellicle may be formed.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae may consist merely of a few short chains of cells or of long, branched chains of cylindrical cells with well-differentiated blastoconidia. Aerobic growth varies from smooth to lacy and wrinkled, white to off-white, creamy and soft. The margin may be entire or irregular.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	v	Trehalose	v
Maltose	v		

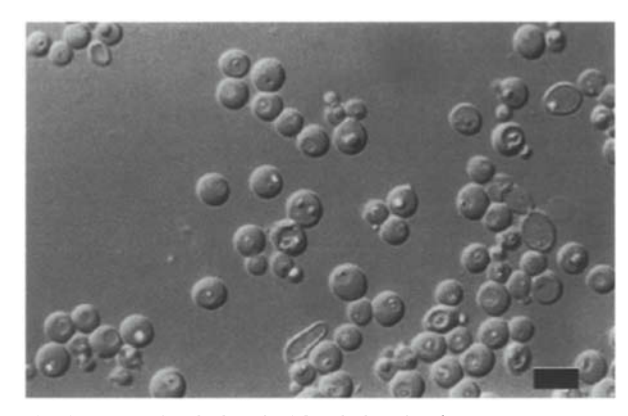


Fig. 257. *C. sake*, CBS 159. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	v
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	v
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	v
5-Keto-D-gluconate	v	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	v
D-Glucuronate	–	10% NaCl/5% glucose	v
Xylitol	v	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	v	Biotin-free	v
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	v	0.01% Cycloheximide	–
Nitrite	–	Growth at 25°C	+
Cadaverine	+	Growth at 30°C	v
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G + C: 37.8–41.0, 10 strains (T_m: Meyer et al. 1975); 39.3, CBS 159; 39.3, CBS 617 (T_m: Kaneko et al. 1977); 40.1, CBS 159; 39.7, JMC 1595 (CBS 5690) (T_m: Suzuki and Nakase 1988b).

Origin of the strains studied: CBS 159, sake-moto; CBS 617, lambic beer (type strain of *Torula lambica*); CBS 1939, beer conduit in brewery, Sweden; CBS 2282, CBS 2283, egg yolk; CBS 2920 (type strain of *C. vanrijiae*), CBS 2921, soil; CBS 5093, grape juice; CBS 5690, frozen salmon (type strain of *C. salmonicola*); CBS 5740, soil; CBS 5957, water, Antarctica; CBS 6716, CBS 6717, CBS 6718, water, Chesapeake Bay, U.S.A.

Type strain: CBS 159.

Comments: Meyer and Phaff (1972) reported a high degree of DNA relatedness between *C. sake* and *C. salmonicola* type strains. Suzuki and Nakase (1988b) obtained similar results. Also, DNA reassociation studies confirmed the identity of CBS 617, CBS 2920, CBS 6716 and CBS 6717 as *C. sake* (Meyer et al. 1975). This study, as well as another study (Kaneko et al. 1977), showed the lack of significant DNA relatedness between *C. sake, C. maltosa* and *C. tropicalis.* Fukazawa et al. (1975) studied fifteen strains of *C. sake* on the basis of their serological properties and proton magnetic resonance spectra and found that the strains could be divided into four groups. Group I included the type strains of *C. sake, C. tropicalis* var. *lambica, C. salmonicola* and *C. vanrijiae.* Group II was represented by the type strains of *C. maltosa, C. cloacae* and *C. subtropicalis.* The type strains of *C. oleophila* and *C. natalensis* were members of Group III and Group IV, respectively. Montrocher (1980) found similar results with an immunoelectrophoretic technique. Both of these investigations concur with the DNA studies. The degree of variability exhibited by the strains included in *C. sake* is indicative that heterogeneity still exists and additional molecular studies are needed to determine the identity of these strains. A new species, *C. fragi,* isolated from a fermenting strawberry, has physiological properties similar to *C. sake,* as well as *C. oleophila* and *C. natalensis,* but it is distinct from them on the basis of DNA relatedness, electrophoretic enzyme patterns and proton magnetic resonance spectra of the cell wall mannans (Suzuki et al. 1991). Kurtzman and Robnett (1997) found *C. sake* and *C. austromarina* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.125. *Candida salmanticensis* (Santa Maria) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)

Synonyms:

Torulopsis salmanticensis Santa María (1963a)

Candida salmanticensis (Santa María) van Uden & H.R. Buckley (1970) nom. inval.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.0–5.0) × (4.0–8.0) μm, and occur singly, in pairs and in clusters (Fig. 258).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of chains of cylindrical cells with verticils of ovoidal blastoconidia. Aerobic growth is white to cream-colored, dull, and smooth with an entire border.

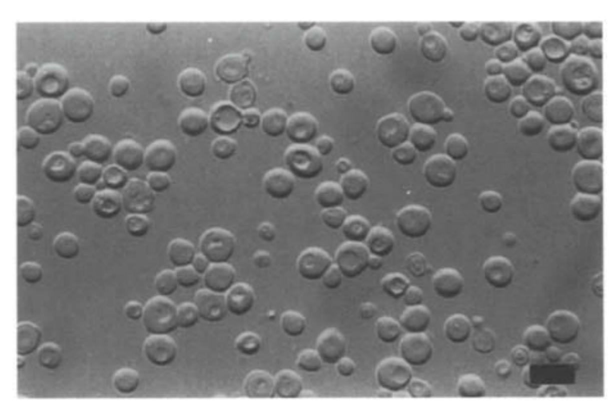


Fig. 258. *C. salmanticensis*, CBS 5121. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	+
Sucrose	+	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	l
Trehalose	+	Galactitol	–
Lactose	–/l	D-Mannitol	l
Melibiose	+	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	l
Inulin	+	D-Gluconate	l
Soluble starch	–	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	n
D-Glucuronate	–	50% Glucose	s
Xylitol	+	10% NaCl/5% glucose	l
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	–
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 45.5, CBS 5121 (BD: Meyer et al. 1984).

Origin of the strain studied: CBS 5121, alpechin (residue from preparation of olive oil).

Type strain: CBS 5121.

64.126. *Candida santamariae* Montrocher (1967)

Synonym:

Candida krusei (Castellani) Berkhout var. *saccharicola* Santa María (1959b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to elongate, (3.5–7.0)×(6.5–12.0) μm. Some single and budding cells are evident, but most of the growth is pseudomycelial. An incomplete pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of extensively branched chains of cylindrical cells, sometimes with blastoconidia singly or in short chains. Aerobic growth is off-white to beige, creamy, smooth to irregular, soft, dull, with a mycelial border.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–/l	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	l	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 36.3, 38.8, 37.3, CBS 4515 (T_m: Nakase and Komagata 1971f; Meyer et al. 1984; Meyer, unpublished data; respectively).

Origin of strains studied: CBS 4515, sugar, Spain.

Type strain: CBS 4515.

Comments: In the original description of *C. santamariae* (Montrocher 1967), sucrose utilization and galactose

fermentation were recorded as positive. Van Uden and Buckley (1970) determined that these results were negative and attributed the difference to the techniques Montrocher used, which were test periods of up to three months. Also, a strain designated as *C. santamariae* var. *membranifaciens* Montrocher (1967) (isolated from an insect, *Hymenochaete rubiginosa*, in France, CBS 5838) was originally described as differing from the species in sucrose utilization, galactose fermentation and pellicle formation. Without studying this strain, van Uden and Buckley questioned its status as a variety since the type strain of *C. santamariae* failed to utilize sucrose and failed to ferment galactose. Meyer et al. (1984) noted that the mol% G+C was significantly higher (46%) for the varietal strain and stated that more studies needed to be done to identify this strain. (The reported value of 46% was an error. It should have been 44.6%. This was the value reported by Stenderup et al. 1972.) Nevertheless, this value is still significantly higher than that of the *C. santamariae* type strain. Other data (Meyer, unpublished data) places the mol% G+C at 37.3–38.5, based on two T_m determinations. These GC values are closer to that of the type strain of *C. santamariae*. DNA reassociation studies will resolve the relationship of these two strains. Since the taxonomic position of *C. santamariae* var. *membranifaciens* remains uncertain, it is not included as a variety of the species at this time. Kurtzman and Robnett (1997) found *C. beechii* and *C. santamariae* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.127. *Candida santjacobensis* C. Ramírez & A. González (1984i)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.0–3.5)×(2.0–5.0) µm, single and in pairs (Fig. 259).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, a few, short pseudohyphae are formed outside the coverslip. Aerobic growth is creamy, off-white, soft and entire.

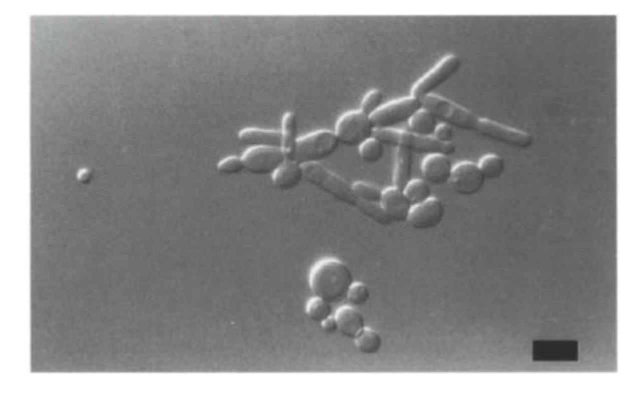


Fig. 259. *C. santjacobensis*, CBS 8183. After 5 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Fermentation:

Glucose	s	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	+	D-Glucitol	l
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	–/l	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	+
D-Glucuronate	+	50% Glucose	–
Xylitol	l	10% NaCl/5% glucose	–
L-Arabinitol	l	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	l
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	w/–
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 48.5, type strain (T_m: Meyer, unpublished data).

Origin of the strain studied: CBS 8183, rotten wood, Chile.

Type strain: CBS 8183.

64.128. *Candida savonica* Sonck (1974)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, cells have various shapes, mostly ovoidal or ellipsoidal and cylindroidal, (2.8–8.4)×(5.6–11.2) µm, and occur singly, in pairs and in short chains. After one month a thick sediment is evident. The sediment appears clumpy, but on shaking becomes homogeneous.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of extensively branched chains of elongate cells with single or short chains of blastoconidia. Aerobic growth is off-white, smooth, butyrous, flat and exhibits a mycelial border.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	+/l
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	l	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	l

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	n
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	l
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	l
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	w
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 48.0, CBS 6563 (T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 6563, birch log, Finland.

Type strain: CBS 6563.

64.129. *Candida schatavii* (Kocková-Kratochvílová & Ondrušová) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis schatavii Kocková-Kratochvílová & Ondrušová (1971)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to elongate, (2.9–5.8)×(3.6–7.2) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with a few ovoidal blastoconidia. Aerobic growth is white to cream-colored, smooth, moist and glistening, and has a fringed margin.

Fermentation:

Glucose	s	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	l	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–/l	Inositol	–
D-Ribose	+	Hexadecane	+/l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	D-Glucosamine (N)[1]	n
Saccharate	n	50% Glucose	–/l
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	l	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	–	Growth at 35°C	+
Cadaverine	+	Growth at 37°C	w/–
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 44.6, 43.7, CBS 6452 (T_m: Meyer et al. 1984; T_m: Meyer, unpublished data, respectively).

Origin of the strain studied: CBS 6452, pileus of a fungus (*Fomitopsis pinicola*).

Type strain: CBS 6452.

64.130. *Candida sequanensis* Saëz & Rodrigues de Miranda (1984)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (2.5–7.5) μm, single and in pairs (Fig. 260).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is white, flat and glossy, with a lobate margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

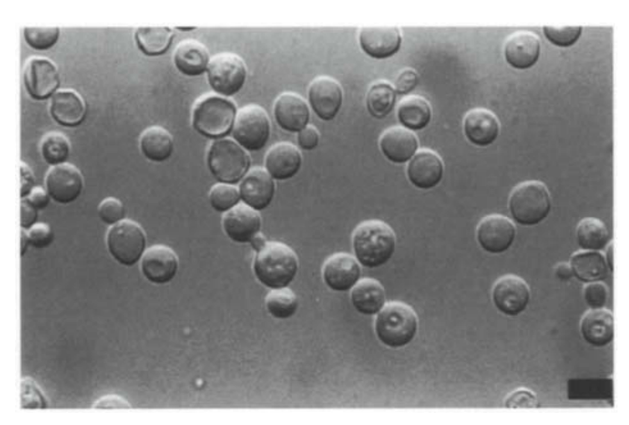


Fig. 260. *C. sequanensis*, CBS 8118. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	l	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 39.6, type strain (T_m: Saëz and Rodrigues de Miranda 1984).

Origin of the strain studied: CBS 8118, oats.

Type strain: CBS 8118.

64.131. *Candida shehatae* H.R. Buckley & van Uden (1967)

This species has three varieties:

***Candida shehatae* H.R. Buckley & van Uden var. *shehatae* (1990)**

***Candida shehatae var. insectosa* Kurtzman (1990b)**

***Candida shehatae var. lignosa* Kurtzman (1990b)**

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (2.0–4.5) × (3.5–6.0) µm, and occur singly, in pairs and short chains (Fig. 261). Pseudohyphae are also present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells with globose to ovoidal blastoconidia. Aerobic growth is white to cream-colored, glistening, and smooth with a reticulated margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	+/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–/l	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–/l	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

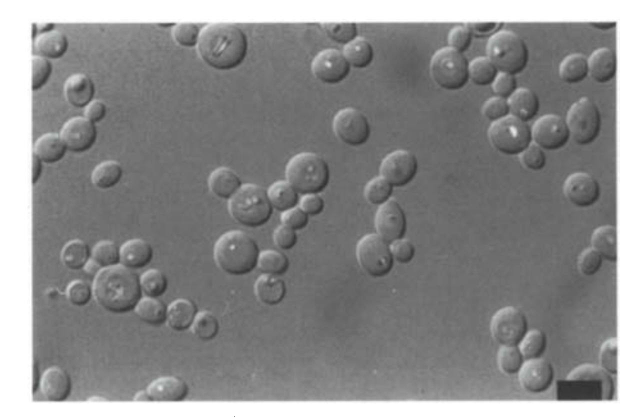


Fig. 261. *C. shehatae* var. *shehatae*, CBS 7261. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	v
Xylitol	+/l	Starch formation	–
L-Arabinitol	–/l	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	+
Nitrite	–	0.1% Cycloheximide	v
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 41.2, one strain (T_m: Nakase and Komagata 1971f); 41.2, one strain (T_m: Vaughan-Martini 1984); 41.2–43.4, 4 strains (T_m: Tengku Zainal Mulok 1988); 43.5–43.7, 3 strains (BD: Kurtzman 1990b).

Supplementary descriptions of *C. shehatae* varieties:

Variety *insectosa*: L-sorbose, –; L-arabinose, –; erythritol, –; L-arabinitol, –; gelatin liquefaction, w; 0.1% cycloheximide, +; mol% G+C: 44.4, 44.6, 2 strains (Kurtzman 1990b).

Variety *lignosa*: L-sorbose, +; L-arabinose, v; galactitol, v; D-gluconate, –; DL-lactate, w; L-arabinitol, –; 0.1% cycloheximide, +; mol% G+C: 44.1, type strain (Kurtzman 1990b).

Origin of the strains belonging to the variety *shehatae*: CBS 5712, rose-hip, Canada; CBS 5813, dead insect-invaded pine tree; CBS 7261, CBS 7263, forest soil, South Africa; CBS 2779, soil, South Africa.

Type strain: CBS 5813.

Origin of the strains belonging to the variety *insectosa*: CBS 4286, an insect, *Leptura maculicornis*; CBS 4287, an insect, *Leptura cerambyciformis*.

Type strain: CBS 4286.

Origin of the strain belonging to the variety *lignosa*: CBS 4705, wood.

Type strain: CBS 4705.

Comments: Vaughan-Martini (1984) showed by DNA reassociation that *C. shehatae* was not the anamorph of *Pichia stipitis*. This was confirmed by Tengku Zainal Mulok (1988) and by Kurtzman (1990b). Kurtzman's studies not only included DNA reassociation, but ribosomal RNA sequence comparisons. He also found that the strains of *C. shehatae* formed three DNA groups with about 50% DNA reassociation between members of the different groups. He designated three varieties, *C. shehatae* var. *shehatae*, var. *insectosa*, and var. *lignosa*, and found that the physiological properties that separate these varieties are the utilization of galactitol and lactate by var. *lignosa* and the weak liquefaction of gelatin by var. *insectosa*. Our results were variable for the utilization of galactitol by var. *lignosa* because the tests are terminated after 3 weeks. If they were allowed to run 4 weeks, latent or negative reactions were noted.

64.132. *Candida silvae* Vidal-Leiria & van Uden (1963)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–4.0)×(3.0–6.0) μm, single and in pairs (Fig. 262). Islets and a ring may be present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of elongate cells with verticils of ovoid blastoconidia. Aerobic growth is white, butyrous, soft, mostly smooth, somewhat glistening and entire.

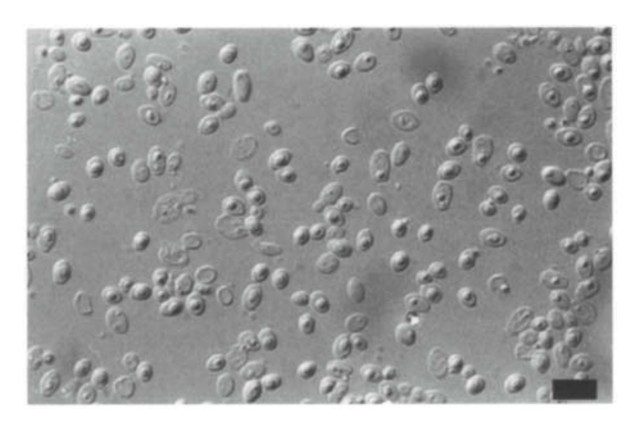


Fig. 262. *C. silvae*, CBS 5498. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+/l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+/l
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–/l
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–/l	Biotin-free	+/l
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	v
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (Suzuki et al. 1994).

Mol% G+C: 39.5, CBS 5498 (T_m: Suzuki et al. 1994); 44.0, CBS 5497 (BD: Meyer et al. 1984).

Origin of the strains studied: CBS 988, yeast cake; CBS 1520, vagina; CBS 2832, sputum; CBS 5497, CBS 5498, horse intestine.

Type strain: CBS 5498.

Comments: Suzuki et al. (1994) described a new species, *C. stellimalicola,* that resembles *C. silvae, C. diversa* and *C. karawaiewii* in physiological and morphological properties. *C. stellimalicola* is differentiated from *C. silvae* on the basis of a vitamin requirement. See Comments for *C. diversa* for more information.

64.133. *Candida silvanorum* van der Walt, van der Klift & D.B. Scott (van der Walt et al. 1971a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.0–3.5)×(2.0–4.0) μm, single, in pairs, small chains and groups.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, well-developed pseudohyphae consist of long chains of slender elongate cells with verticils of usually triangular blastoconidia. Aerobic growth is white to cream-colored, butyrous with a raised, wrinkled central area and an entire or fringed margin.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	s
Sucrose	s	Trehalose	s
Maltose	s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–/l
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 40.5, 41.7, CBS 6274 (T_m: van der Walt et al. 1971a; BD: Meyer et al. 1984, respectively).

Origin of the strain studied: CBS 6274, beetle-infested tree, South Africa.

Type strain: CBS 6274.

64.134. *Candida silvatica* (van der Walt, van der Klift & D.B. Scott) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis silvatica van der Walt, van der Klift & D.B. Scott (1971b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (2.0–4.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is white to slightly cream-colored, butyrous, smooth and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	n
L-Arabinitol	–	Starch formation	–
Arbutin	–/l	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 55.6, CBS 6277 (T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 6277, beetle-infested tree, South Africa.

Type strain: CBS 6277.

64.135. *Candida silvicultrix* van der Walt, D.B. Scott & van der Klift (1972)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to short-ovoidal, (2.5–6.0)×(4.5–7.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, abundant development of pseudohyphae is present. Pseudohyphae consist of branched chains of elongate cells with dense verticils of blastoconidia. Aerobic growth is off-white, butyrous, smooth, and shiny with both an entire and a fringed border.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	+
Sucrose	+	Trehalose	–
Maltose	s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	+
Butane 2,3 diol	+	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 36.6, CBS 6269 (T_m: Meyer et al. 1984).

Origin of the strain studied: CBS 6269, beetle-infested tree, South Africa.

Type strain: CBS 6269.

64.136. *Candida solani* Lodder & Kreger-van Rij (1952)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, sometimes slightly elongated, (3.5–5.5)×(6.0–11.0) μm, single, in pairs and short chains (Fig. 263).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with ovoidal blastoconidia. Aerobic growth is off-white, butyrous, smooth, soft, flat, and dull with a slightly irregular edge.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

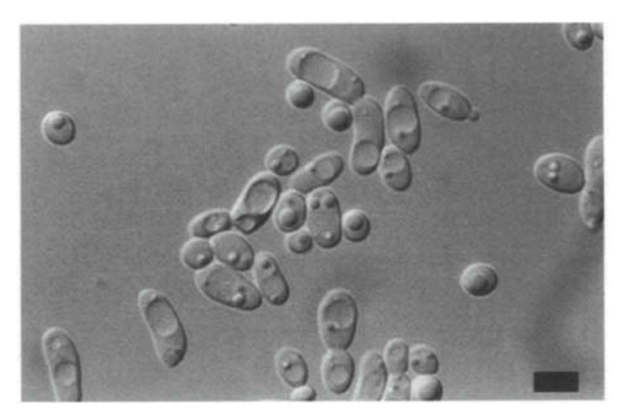


Fig. 263. *C. solani*, CBS 1908. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	+	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (Yamada and Kondo 1972a).

Mol% G + C: 41.9, CBS 1908 (T_m: Meyer and Phaff 1972).

Origin of the strain studied: CBS 1908, potato-starch mill.

Type strain: CBS 1908.

64.137. *Candida sonorensis* (M.W. Miller, Phaff, Miranda, Heed & Starmer) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis sonorensis M.W. Miller, Phaff, Miranda, Heed & Starmer (1976a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (2.5–5.0) × (4.0–6.0) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. However, there are small clusters of chained cells. Aerobic growth is off-white, butyrous, soft, smooth, shiny and entire to slightly lobed.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	+
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	l
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–/l	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (J.-D. Lee and Komagata 1980a).

Mol% G + C: 36.0–36.1, 2 strains (BD: Miller et al. 1976a).

Origin of the strains studied: CBS 6792, CBS 6793, cacti, Mexico and USA.

Type strain: CBS 6792.

64.138. *Candida sophiae-reginae* C. Ramírez & A. González (1984g)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are long-ovoidal, ellipsoidal or cylindrical, (2.0–4.5) × (6.0–10.0) µm, single, in pairs and short chains (Fig. 264).

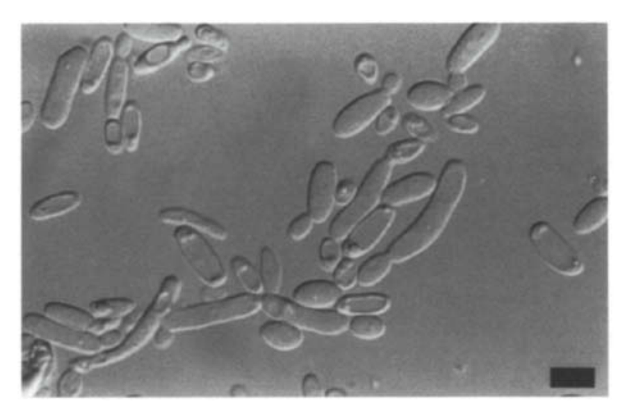


Fig. 264. *C. sophiae-reginae*, CBS 8175. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, well-developed pseudohyphae with branched chains of cells possessing clusters of oval blastoconidia are present. Aerobic growth is white, butyrous, and glistening. The margin is entire to irregular.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	l
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.
Mol% G+C: 39.3, CBS 8175 (T_m: Tengku Zainal Mulok 1988).

Origin of the strain studied: CBS 8175, rotten wood, Chile.

Type strain: CBS 8175.

64.139. *Candida sorbophila* (Nakase) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis sorbophila Nakase (1975)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (2.0–3.5)×(3.0–4.0) μm, single and in pairs (Fig. 265).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent or consist of short, branched chains of ovoid cells. Aerobic growth is white, butyrous, smooth, glistening and entire.

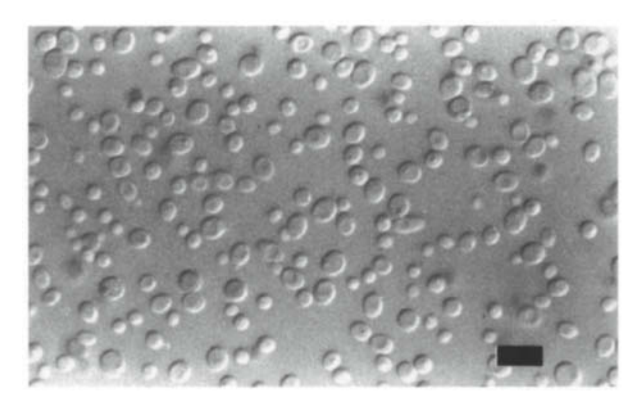


Fig. 265. *C. sorbophila*, CBS 6739. After 4 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+/l
Sucrose	–	Glycerol	l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	l	Succinate	+
L-Arabinose	v	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	+/l
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	l	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–/l
Propane 1,2 diol	–	Pyridoxine-free	–
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	–	Growth at 35°C	+
Cadaverine	+	Growth at 37°C	v
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 50.0, CBS 6739 (T_m: Nakase 1975).

Origin of the strains studied: CBS 6739, ion-exchange resin in a guanine monophosphate factory, Japan; CBS 7062, unknown, Switerland; CBS 7266, soil, South Africa.

Type strain: CBS 6739.

64.140. *Candida sorboxylosa* Nakase (1971c)

Growth in glucose-yeast extract-peptone broth: After 3 days at 25°C, the cells are ovoidal to long-ovoidal, (3.0–6.0)×(4.0–8.4) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of ovoidal cells with clusters of blastoconidia. Aerobic growth is off-white, butyrous, smooth, and soft with an entire to an irregular margin.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	+
Saccharate	–	50% Glucose	v
D-Glucuronate	–	10% NaCl/5% glucose	l
Xylitol	+	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	+
Propane 1,2 diol	–	Pyridoxine-free	–
Butane 2,3 diol	–	0.01% Cycloheximide	–
Nitrite	–	Growth at 35°C	+
Cadaverine	+	Growth at 37°C	v
Creatinine	–	Growth at 40°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 39.8, 39.9, 2 strains (T_m: Nakase 1971c); 40.7, CBS 2120, CBS 2121 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 2120, fermenting cacao; CBS 2121, palm wine; CBS 6378, pineapple; CBS 8042, *Euphorbia* species.

Type strain: CBS 6378.

64.141. *Candida spandovensis* (Henninger & Windisch) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:
Torulopsis spandovensis Henninger & Windisch (1976b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to short-ovoidal, (2.5–3.5)×(3.0–4.5) μm, single and in pairs (Fig. 266).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent or short chains of budding cells are present. Aerobic growth is white, butyrous, shiny, convex and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	s/–
Sucrose	s	Trehalose	–
Maltose	–		

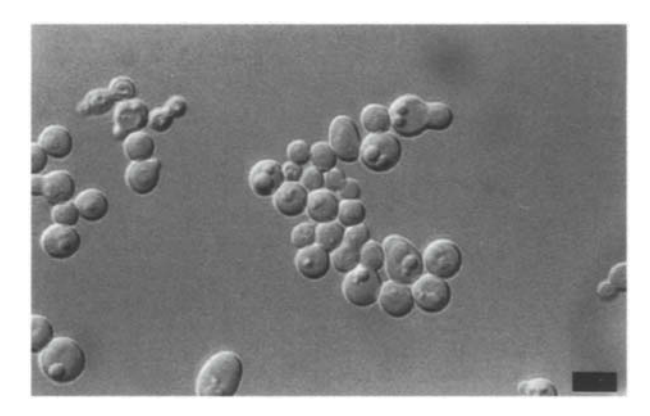


Fig. 266. *C. spandovensis*, CBS 5511. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	l
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+
Xylitol	+	10% NaCl/5% glucose	l
L-Arabinitol	v	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	–
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 52.7, CBS 6875 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 5511, CBS 6875, beer, Germany.

Type strain: CBS 6875.

64.142. *Candida stellata* (Kroemer & Krumbholz) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Saccharomyces stellatus Kroemer & Krumbholz (Krumbholz 1931)
Torulopsis stellata (Kroemer & Krumbholz) Lodder (1932)
Cryptococcus stellatus (Kroemer & Krumbholz) Skinner (1947a)
Saccharomyces bacillaris Kroemer & Krumbholz (Krumbholz 1931)
Torulopsis bacillaris (Kroemer & Krumbholz) Lodder (1932)
Cryptococcus bacillaris (Kroemer & Krumbholz) Skinner (1947a)
Brettanomyces italicus Verona & Florenzano (1947)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (4.0–5.5)×(4.0–6.0) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is white, butyrous, soft, glossy and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	+/l
Sucrose	+	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–/l	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	–
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+/l
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–
Nitrite	–	Growth at 30°C	+
Cadaverine	–	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 8 (Yamada and Kondo 1972a).

Mol% G+C: 42.0, one strain (T_m: Nakase and Komagata 1971d).

Origin of the strains studied: CBS 157, wine grapes; CBS 1713, wine; CBS 1779, tea-beer; CBS 4729, fruit fly (*Drosophila pinicola*); CBS 6100, unknown.

Type strain: CBS 157.

64.143. *Candida succiphila* J.-D. Lee & Komagata (1980b)

Synonym:

Candida methanolophaga Kumamoto & Yamamoto (Kumamoto et al. 1986)

Growth in glucose-yeast extract-peptone broth: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.0–4.5)×(3.0–6.0) µm, single, in pairs and short chains (Fig. 267).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is white to slightly cream-colored, smooth, butyrous, soft, glistening and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	–	Trehalose	+
Maltose	–		

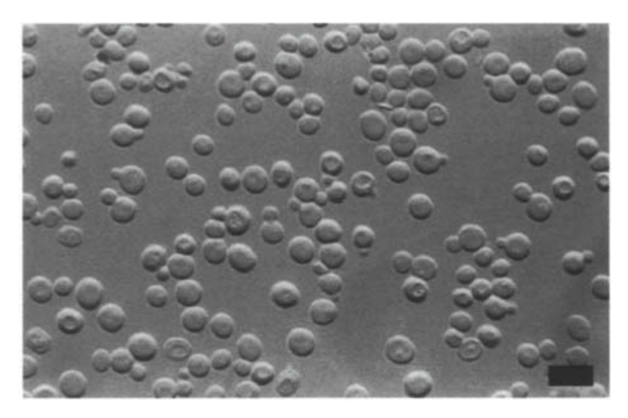


Fig. 267. *C. succiphila*, CBS 7297. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	+
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+/l
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–/l

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	–
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	+	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–/l
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+/l
Cadaverine	–	Growth at 37°C	+/w
Creatinine	–	Growth at 40°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 7 (J.-D. Lee and Komagata 1980a); 7 (C.-F. Lee et al. 1994b).

Mol% G + C: 40.9, CBS 8003 (T_m: J.-D. Lee and Komagata 1980a); 41.2, CBS 7297 (T_m: Kumamoto et al. 1986); 40.0, CBS 8003; 39.5, CBS 7297 (HPLC: C.-F. Lee et al. 1994b).

Origin of the strains studied: CBS 8003, sap of a peach tree, Japan; CBS 7297, oil field, Japan.

Type strain: CBS 8003.

Comments: C.-F. Lee et al. (1994b) found identical chromosomal DNA banding patterns and 90% DNA relatedness between the type strains of *C. methanolophaga* and *C. succiphila* to confirm their conspecificity.

64.144. *Candida suecica* Rodrigues de Miranda & Norkrans (1968)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, long ovoidal, ellipsoidal or cylindrical, (2.2–3.6) × (5.8–13.7) μm, single, in pairs and short chains (Fig. 268). Pseudohyphae are present.

Growth in yeast nitrogen base + 0.5% glucose: After 3 days at 25°C, a delicately wrinkled pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, abundant pseudohyphae are present and consist of branched chains of cylindrical cells, many with verticils of ovoidal blastoconidia. Aerobic growth is white, smooth and wrinkled, soft, and dry with a lobed margin.

Fermentation:

Glucose	–/l	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–/l
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–/l	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	–/l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	–	Citrate	+/l
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	–
5-Keto-D-gluconate	n	Ethylamine	–
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	v	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	–	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 42.7, 42.4, CBS 5724 (T_m: Meyer et al. 1984; BD: Kurtzman 1987a, respectively).

Origin of the strain studied: CBS 5724, sea water, Sweden.

Type strain: CBS 5724.



Fig. 268. *C. suecica*, CBS 5724. After 5 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

64.145. *Candida tanzawaensis* Nakase & M. Itoh (Nakase et al. 1988b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (1.0–2.0)×(1.5–4.0) µm, and occur singly or in pairs. Short, branched chains of cells are also present. Other cells are long ovoidal or elongate (10 µm and longer), and slender; some are branched. There is abundant pseudohyphal development. A pellicle appears to have dropped from the surface and the liquid is flocculant.

Growth in yeast nitrogen base + 0.5% glucose: After 3 days at 25°C, a wrinkled pellicle is present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of long, branched chains of cells with globose to ovoidal blastoconidia occurring singly or in small verticils. Septate hyphae may be present. Aerobic growth is white, dull, and irregular. The margin is fringed.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	ws
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	l
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Nakase et al. 1988b).

Mol% G + C: 45.1, 44.1, CBS 7422 (T_m and HPLC, respectively: Nakase et al. 1988b.

Origin of the strains studied: CBS 7422, moss (*Polytrichum commune*), Mt. Tanzawa, Japan.

Type strain: CBS 7422.

Comments: In the original description of *C. tanzawaensis*, Nakase et al. (1988b) reported D-arabinose positive or latent, ribose variable, galactitol, L-rhamnose and soluble starch negative or weakly positive, growth on 50% glucose weakly positive and growth at 35°C positive. These investigators recognized the physiological similarities between *C. tanzawaensis*, *C. kruisii* and *C. oleophila* and found the mol% G + C for *C. oleophila* (41.7%) lower than that of *C. tanzawaensis* (45.1%) and *C. kruisii* (45.4%). DNA reassociation confirmed that these species are distinct.

64.146. *Candida tenuis* Diddens & Lodder (1942)

Synonym:
Mastigomyces philippovii Imshenetskii & Kriss (1933)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to cylindroidal or elongate, (1.5–3.5)×(2.0–7.0) µm, single, in pairs, chains and clusters (Fig. 269). Pseudohyphae may be present.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells, sometimes with verticils of ovoidal

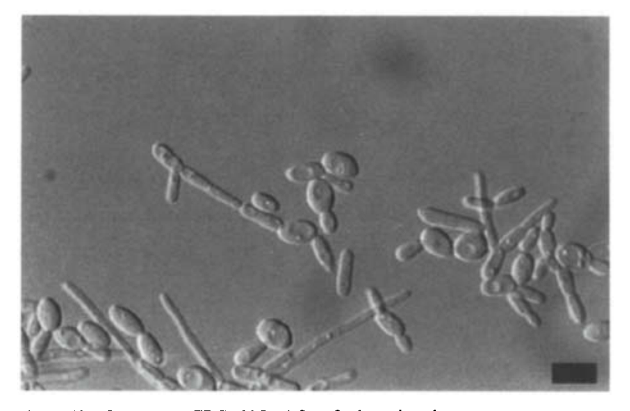


Fig. 269. *C. tenuis*, CBS 615. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 µm.

blastoconidia. Septate hyphae may be present. Aerobic growth is off-white, creamy, lacy to wrinkled, with a fringed border.

Fermentation:

Glucose	v	Lactose	–
Galactose	v	Raffinose	–
Sucrose	–/s	Trehalose	v
Maltose	–/s		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+/l	D-Mannitol	+
Melibiose	–	D-Glucitol	+/l
Raffinose	–/l	α-Methyl-D-glucoside	+
Melezitose	+/l	Salicin	+
Inulin	–	D-Gluconate	+/l
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–/l
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	v
Saccharate	–	50% Glucose	–/l
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+/l	Starch formation	–
L-Arabinitol	v	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–/l	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	v
Nitrite	–	0.1% Cycloheximide	v
Cadaverine	+	Growth at 30°C	+/w
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G + C: 44.0, CBS 615 (T_m: Stenderup and Bak 1968); 43.8, one strain (Miranda et al. 1982); 43.2, AJ 4667 (T_m: Nakase and Komagata 1971f); 44.4, CBS 7047 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 615, CBS 2308, CBS 2309, CBS 2885, CBS 4113, CBS 4285, CBS 4435, bark beetles; CBS 7047 (neotype of *Mastigomyces philippovii*), litter of coniferous forest.

Type strain: CBS 615.

Comments: Kurtzman and Robnett (1997) examined the LSU rRNA gene sequence of CBS 7047 (neotype of *Mastigomyces philippovii*) and found it to have only one nucleotide difference from the type strain of *C. tenuis*.

64.147. *Candida tepae* Grinbergs (1967)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C the cells are globose to ovoidal, (3.0–5.0) × (4.0–9.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of densely branched chains of ovoidal cells. Aerobic growth is off-white, butyrous, smooth, soft, mostly entire, but with a few tufts of pseudomycelical development.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	l	Methanol	–
L-Sorbose	l	Ethanol	l
Sucrose	+	Glycerol	l
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	l	Galactitol	–
Lactose	–	D-Mannitol	l
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–/l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	l	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	–
5-Keto-D-gluconate	n	Ethylamine	–
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	l	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	–	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 41.0, CBS 5115 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 5115, rotten wood (*Laurelia philippiana*).

Type strain: CBS 5115.

Comments: Kurtzman and Robnett (1997) found *C. tepae, C. antillancae* and *C. bondarzewiae* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, thus indicating the three taxa to be conspecific.

64.148. *Candida torresii* (van Uden & Zobell) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis torresii van Uden & Zobell (1962)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal to long-ovoidal, (1.5–2.5)×(3.5–7.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is off-white, smooth, butyrous and entire.

Fermentation:

Glucose	+/s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	+/s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	l	Methanol	–
L-Sorbose	+/l	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	–/l
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	s
Xylitol	+	10% NaCl/5% glucose	+
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	v	Biotin-free	v
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 52.0, AJ 4937 (T_m: Nakase and Komagata 1971f); 51.7, 51.7, CBS 5152 (BD: Meyer et al. 1984; T_m: Ribeiro 1995, respectively).

Origin of the strains studied: CBS 5152, sea water, Torres Strait, Australia; CBS 6035, tunnels of bark beetles in *Ficus sycomorus*, South Africa.

Type strain: CBS 5152.

64.149. *Candida tropicalis* (Castellani) Berkhout (1923)

Synonyms:

Oidium tropicale Castellani (1910)
Monilia tropicalis (Castellani) Castellani & Chalmers (1913)
Endomyces tropicalis Castellani (1911) [non *Endomyces tropicalis* Acton 1919]
Atelosaccharomyces tropicalis (Castellani) Froilano de Mello (Froilano de Mello and Gonzaga Fernandes 1918)
Myceloblastanon tropicale (Castellani) Ota (1928)
Castellania tropicalis (Castellani) Dodge (1935)
Procandida tropicalis (Castellani) Novák & Zsolt (1961)
Monilia candida Bonorden (1851)
Monilia candida Hansen (1888a)
Saccharomyces linguae-pilosae Lucet (1901)
Cryptococcus linguae-pilosae (Lucet) Gedoelst (1902)
Myceloblastanon linguae-pilosae (Lucet) Ota (1928)
Torulopsis linguae-pilosae (Lucet) de Almeida (1933)
Castellania linguae-pilosae (Lucet) Dodge (1935)
Monilia bonordenii Vuillemin (1911)
Candida bonordenii (Vuillemin) Basgal (1931)
Endomyces paratropicalis Castellani (1911)
Monilia paratropicalis (Castellani) Castellani & Chalmers 1913)
Atelosaccharomyces paratropicalis (Castellani) Froilano de Mello (Froilano de Mello and Gonzaga Fernandes 1918)
Myceloblastanon paratropicale (Castellani) Ota (1928)
Candida paratropicalis (Castellani) Basgal (1931)
Mycocandida paratropicalis (Castellani) Guerra (1935)
Castellania paratropicalis (Castellani) Dodge (1935)
Candida paratropicalis J.G. Baker, Salkin, Pincus & D'Amato (1981) [non *Candida paratropicalis* (Castellani) Basgal (1931)]
Endomyces bronchialis Castellani (1912b)
Monilia bronchialis Castellani (Castellani and Chalmers 1913)
Myceloblastanon bronchiale (Castellani) Ota (1928)
Candida bronchialis (Castellani) Basgal (1931)
Castellania bronchialis (Castellani) Dodge (1935)
Endomyces entericus Castellani (1912b)
Monilia enterica (Castellani) Castellani & Chalmers (1913)
Myceloblastanon entericum (Castellani) Ota (1928)
Candida enterica (Castellani) de Almeida (1933)
Castellania enterica (Castellani) Dodge (1935)
Monilia faecalis (Castellani) Castellani & Chalmers (1913)
Myceloblastanon faecalis (Castellani) Ota (1928)
Castellania faecalis (Castellani) Dodge (1935)
Endomyces insolitus Castellani (1912a)
Monilia insolita (Castellani) Castellani & Chalmers (1913)
Myceloblastanon insolitum (Castellani) Ota (1928)
Candida insolita Redaelli (Graziano 1930)
Castellania insolita (Castellani) Dodge (1935)
Endomyces niveus Castellani (1912a)
Monilia nivea (Castellani) Castellani & Chalmers (1913)
Myceloblastanon niveum (Castellani) Ota (1928)
Candida nivea (Castellani) Basgal (1931)
Castellania nivea (Castellani) Dodge (1935)
Endomyces burgessi Castellani (1913)
Monilia burgessi Castellani (Castellani and Chalmers 1913)
Castellania burgessi (Castellani) Dodge (1935)
Endomyces perryi Castellani (1913)
Parendomyces perryi (Castellani) Dodge (1935)
Parasaccharomyces candidus Anderson (1917)
Myceloblastanon candidum Ota (1928)
Endomyces cruzi Froilano de Mello & Paes (1918)
Zymonema cruzi (Froilano de Mello & Paes) Dodge (1935)
Monilia metatropicalis Castellani (Castellani and Chalmers 1919)
Castellania metatropicalis (Castellani & Chalmers) Dodge (1935)
Monilia pseudobronchialis Castellani (Castellani and Chalmers 1919)
Monilia accraensis Macfie (1921)
Candida vulgaris Berkhout (1923)
Geotrichoides vulgaris (Berkhout) Langeron & Talice (1932)
Blastodendrion irritans Mattlet (1926)
Parasaccharomyces irritans (Mattlet) Dodge (1935)
Blastodendrion kayongosi Mattlet (1926)
Cryptococcus kayongosi (Mattlet) Nannizzi (1934)
Torulopsis tonsillae Carnevale-Ricci (1926)

Monilia issavi Mattlet (1926)
Syringospora issavi (Mattlet) Dodge (1935)
Mycotorula interdigitalis Redaelli *apud* Flarer (1931)
Mycotorula dimorpha Redaelli & Ciferri (Ciferri and Redaelli 1935)
Syringospora dimorpha (Redaelli & Ciferri) Dodge & Moore (1936)
Mycotorula trimorpha Redaelli & Ciferri (Ciferri and Redaelli 1935)
Mycotoruloides trimorpha (Redaelli & Ciferri) Dodge & Moore (1936)
Monilia aegyptiaca Khouri (1932)
Castellania aegyptiaca (Khouri) Dodge (1935)
Monilia argentina Vivoli, Avellaneda & de Bardessi (1932)
Mycotoruloides argentina (Vivoli, Avellaneda & de Bardessi) Dodge (1935)
Cryptococcus mattleti Nannizzi (1934)
Castellania accraensis (Macfie & Ingram) Dodge (1935)
Monilia murmanica Plevako and Cheban (1935)
Parasaccharomyces talicei Dodge (1935)
Pseudomonilia miso Mogi (1942)
Mycotorula japonica Yamaguchi (1943)
Candida benhamii Novák & Vitéz (1964)
Candida citrica Furukawa, Matsuyoshi, Minoda & K. Yamada (1977)
Candida bimundalis Wickerham & Santa María var. *chlamydospora* Nowakowska-Waszczuk & Pietka (1983)
Torulopsis candida (Saito) Lodder var. *nitratophila* Nowakowska-Waszczuk & Pietka (1983)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C the cells are subglobose to ovoidal, (3.5–7.0)×(5.5–10.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells with blastoconidia formed singly or in verticils; septate hyphae are usually present. Aerobic growth is white, smooth, butyrous, and soft with a fringed border.

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	v	Trehalose	+/s
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	v	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+/l	Ribitol	+/l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	v
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+/l
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	Ethylamine	+
Saccharate	n	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	+/l
Xylitol	+/l	10% NaCl/5% glucose	l
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–/l	Biotin-free	v
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 34.9, one strain (T_m: Stenderup and Bak 1968); 34.9, UCD-FST 60-31 (T_m: Meyer and Phaff 1969); 35.0, one strain (T_m: Nakase and Komagata 1968a); 34.4–35.4, 3 strains (T_m: Nakase and Komagata 1971f) 36.1, CBS 94; 35.1, CBS 6320; 35.9, CBS 5701 (T_m: Meyer et al. 1975); 34.1, IFO 0006; 34.4, ATCC 750 (T_m: Kaneko et al. 1977); 33.2, GSU 59; 34.6, GSU 372 and 446 (T_m: Ahearn et al. 1977); 35.6, 2 strains (T_m: Su and Meyer 1991).

Origin of the strains studied: CBS 94, human; CBS 642, unknown; CBS 643, kefyr; CBS 2310 (type strain of *C. vulgaris*), unknown; CBS 2311, unknown, from O. Winge; CBS 2313 (type strain of *Mycotorula dimorpha*, interdigital infection); CBS 2314 (*Mycotorula trimorpha*), child's feces; CBS 2317, fodder yeast; CBS 2318, sprue in Puerto Rico (Ashford 1917); CBS 2320, unknown, from Dessy; CBS 4913, unknown, from Sawai as *Mycotorula japonica*; CBS 5701 (type strain of *C. benhamii*), infected nail; CBS 6320 (citric-acid producer); CBS 6361, CBS 6362, blood (Ahearn et al. 1977); CBS 6400, soil, Japan; CBS 6628, CBS 6632, unknown; CBS 6719, water; CBS 7097, human, Japan; CBS 8072 (type strain of *C. paratropicalis* Baker et al. 1981), blood; CBS 8250 (type strain of *C. bimundalis* var. *chlamydospora*), CBS 8252 (type strain of *Torulopis candida* var. *nitratophila*), fermentation vats, Poland.

Type strain: CBS 94.

Comments: Meyer et al. (1975) and Kaneko et al. (1977) performed DNA reassociation experiments to confirm that *C. tropicalis, C. maltosa* and *C. sake* are distinct species. These species are physiologically similar, but can be separated on growth at 35°C (*C. sake*, negative; *C. tropicalis, C. maltosa*, positive) and the assimilation of soluble starch (*C. maltosa, C. sake*, negative; *C. tropicalis*, positive).

64.150. *Candida tsuchiyae* Nakase & M. Suzuki (1985d)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (3.5–5.0)×(4.5–6.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is white, smooth, butyrous and entire.

Fermentation:

Glucose	+	Lactose	−
Galactose	−	Raffinose	s
Sucrose	+	Trehalose	+
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	−
Galactose	−	Methanol	−
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	−
Cellobiose	−	Ribitol	+
Trehalose	+	Galactitol	−
Lactose	−	D-Mannitol	+
Melibiose	−	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	−
Inulin	−	D-Gluconate	+
Soluble starch	+	DL-Lactate	−
D-Xylose	−	Succinate	+
L-Arabinose	−	Citrate	+
D-Arabinose	−	Inositol	−
D-Ribose	−	Hexadecane	−
L-Rhamnose	−	Nitrate	−
D-Glucosamine	+	Vitamin-free	−

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	−	Ethylamine	+
Saccharate	−	D-Glucosamine (N)[1]	−
D-Glucuronate	−	50% Glucose	+
Xylitol	l	10% NaCl/5% glucose	n
L-Arabinitol	−	Starch formation	−
Arbutin	−	Urease	−
Propane 1,2 diol	−	Biotin-free	−
Butane 2,3 diol	n	Pyridoxine-free	+
Nitrite	−	0.1% Cycloheximide	+
Cadaverine	+	Growth at 25°C	+
Creatinine	−	Growth at 30°C	−

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Nakase and Suzuki 1985d).

Mol% G+C: 47.4, 47.6, 2 strains (T_m: Nakase and Suzuki 1985d).

Origin of the strain studied: CBS 7195, moss, Japan.

Type strain: CBS 7195.

64.151. *Candida utilis* (Henneberg) Lodder & Kreger-van Rij (1952)

See *Pichia jadinii*: p. 314

64.152. *Candida vaccinii* Tokuoka, Ishitani, S. Goto & Komagata (1987)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are predominantly globose with some that are slightly ovoidal, (2.0–5.0 μm). Short chains are more frequent than single cells (Fig. 270).

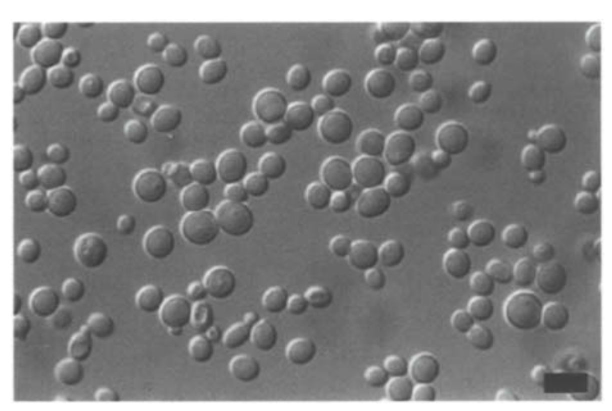


Fig. 270. *C. vaccinii*, CBS 7318. After 3 days in glucose–yeast extract–peptone at 25°C. Bar = 5 μm.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is off-white to beige, smooth and entire.

Fermentation:

Glucose	+	Lactose	−
Galactose	−	Raffinose	−/s
Sucrose	+	Trehalose	−
Maltose	−		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	−
Galactose	+/l	Methanol	−
L-Sorbose	+	Ethanol	−
Sucrose	+	Glycerol	+
Maltose	−	Erythritol	−
Cellobiose	+	Ribitol	−
Trehalose	−	Galactitol	−
Lactose	−	D-Mannitol	+
Melibiose	−	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	−
Melezitose	−	Salicin	+
Inulin	−	D-Gluconate	+
Soluble starch	−	DL-Lactate	−
D-Xylose	−	Succinate	+
L-Arabinose	−	Citrate	+
D-Arabinose	−	Inositol	−
D-Ribose	−	Hexadecane	w
L-Rhamnose	−	Nitrate	+
D-Glucosamine	−	Vitamin-free	−

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	l	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	−
Saccharate	−	50% Glucose	+
D-Glucuronate	−	10% NaCl/5% glucose	+
Xylitol	l	Starch formation	−
L-Arabinitol	−	Urease	−
Arbutin	+	Biotin-free	n
Propane 1,2 diol	−	Pyridoxine-free	n
Butane 2,3 diol	−	0.01% Cycloheximide	l
Nitrite	+	0.1% Cycloheximide	−
Cadaverine	+	Growth at 37°C	+/w
Creatinine	−	Growth at 40°C	−
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Tokuoka et al. 1987).

Mol% G+C: 52.0, CBS 7318 (T_m: Tokuoka et al. 1987).

Origin of the strain studied: CBS 7318, flower of blueberry (*Vaccinium* sp.), Japan.

Type strain: CBS 7318.

Comments: This species is phenotypically similar to *C. magnoliae*, but they differ by ca. 8 mol% G+C. No other characteristic seems to distinguish these two species. See comments for *C. magnoliae*. Also, *C. vaccinii* shares properties in common with *C. etchellsii* and *C. versatilis*.

64.153. *Candida valdiviana* Grinbergs & Yarrow (1970a)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (2.0–4.0)×(4.0–6.5) μm, single and in pairs (Fig. 271).

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae consist of branched chains of elongate cells with verticils of ovoidal blastoconidia. Aerobic growth is white, creamy, smooth, glistening and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	l
Sucrose	+	Glycerol	+/l
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–/l
Trehalose	+	Galactitol	–/l
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+/l	Salicin	+
Inulin	–	D-Gluconate	+/l
Soluble starch	–	DL-Lactate	–/l
D-Xylose	+	Succinate	+
L-Arabinose	–/l	Citrate	+
D-Arabinose	–/l	Inositol	+
D-Ribose	–	Hexadecane	l
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+/l	Vitamin-free	–

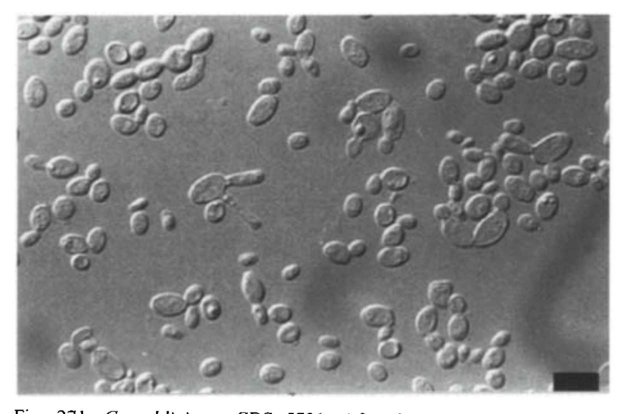


Fig. 271. *C. valdiviana*, CBS 5721. After 3 days in glucose–yeast extract–peptone at 25°C. Bar = 5 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	+
D-Glucuronate	–	50% Glucose	–
Xylitol	–	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	–	Biotin-free	–
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 55.3, CBS 5721 (BD: Meyer et al. 1984).

Origin of the strains studied: CBS 5721, sputum, Chile; CBS 6247, insect frass, Spain.

Type strain: CBS 5721.

64.154. *Candida valida* (Leberle) van Uden & H.R. Buckley (1970)

See *Pichia membranifaciens*: p. 319

64.155. *Candida vanderwaltii* (Vidal-Leiria) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis vanderwaltii Vidal-Leiria (1966b)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (2.0–3.0)×(4.0–6.0) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are usually absent, sometimes short, dense chains of ovoidal cells are present. Aerobic growth is off-white to cream-colored, shiny, soft, smooth and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	–/l	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	l
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	l	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	–	Biotin-free	+
Propane 1,2 diol	–	Pyridoxine-free	–
Butane 2,3 diol	l	0.1% Cycloheximide	+
Nitrite	+	Growth at 30°C	+
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 49.7, CBS 5524 (T_m: Stenderup et al. 1972).

Origin of the strain studied: CBS 5524, winery, South Africa.

Type strain: CBS 5524.

64.156. *Candida vartiovaarae* (Capriotti) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)

Synonyms:

Torulopsis vartiovaarae (as *T. vartiovaarai*) Capriotti (1961e)

Candida vartiovaarae (Capriotti) van Uden & H.R. Buckley (1970) nom. inval.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.0–5.0)×(5.5–8.0) µm, single and in pairs (Fig. 272). Van Uden and Buckley (1970) reported that a pellicle may be formed. This was not confirmed.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are sometimes formed, and consist of branched chains of elongate to cylindrical cells, sometimes with dense verticils of subglobose to ovoidal blastoconidia. Aerobic growth is off-white to cream-colored, somewhat dull, soft, smooth and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	+	Trehalose	–
Maltose	v		

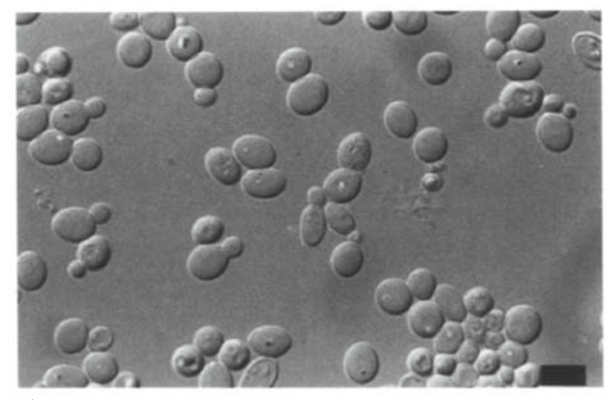


Fig. 272. *C. vartiovaarae*, CBS 4289. After 3 days in glucose–yeast extract–peptone at 25°C. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	+	Biotin-free	+
Propane 1,2 diol	+	Pyridoxine-free	+
Butane 2,3 diol	+	0.01% Cycloheximide	–
Nitrite	+	Growth at 25°C	+
Cadaverine	+	Growth at 30°C	v
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 48.8, CBS 4289 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 4289 and CBS 4290, soil, Finland; CBS 6720, water, U.S.A.; CBS 2895, cider, U.K.

Type strain: CBS 4289.

64.157. *Candida versatilis* (Etchells & T.A. Bell) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonyms:

Brettanomyces versatilis Etchells & T.A. Bell (1950b)

Torulopsis versatilis (Etchells & T.A. Bell) Lodder & Kreger-van Rij (1952)

Torulopsis anomala Lodder & Kreger-van Rij (1952)

Torulopsis mannitofaciens Onishi & T. Suzuki (1969a)

Candida mannitofaciens (Onishi & T. Suzuki) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Torulopsis halophilus Onishi (1957) nom. inval.

Candida halophila Yarrow & S.A. Meyer (1978)

Candida rhodohalophila Mori (Suzuki et al. 1992) nom. nud.

Debaryomyces tamarii Y. Ohara & Nonomura (1954c)

Pichia tamarii (Y. Ohara & Nonomura) Campbell (1973)

Torulaspora tamarii (Y. Ohara & Nonomura ex van der Walt & E. Johannsen) van der Walt & E. Johannsen (1975a)

Debaryozyma tamarii (Y. Ohara & Nonomura ex van der Walt & E. Johannsen) van der Walt & E. Johannsen (1978)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, 3.0–6.0 µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is off-white to beige, glistening soft, smooth and entire.

Fermentation:

Glucose	s	Lactose	–/s
Galactose	s	Raffinose	–/s
Sucrose	v	Trehalose	v
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+/l
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	–/l
Trehalose	+	Galactitol	–
Lactose	–/l	D-Mannitol	+
Melibiose	v	D-Glucitol	–
Raffinose	v	α-Methyl-D-glucoside	–/l
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	–/l
Soluble starch	–	DL-Lactate	v
D-Xylose	–/l	Succinate	+/l
L-Arabinose	–/l	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	–	D-Glucosamine (N)[1]	–
Saccharate	–	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	–/l	Starch formation	–
L-Arabinitol	–/l	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–/l	0.01% Cycloheximide	v
Nitrite	+	0.1% Cycloheximide	v
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	v
L-Lysine	+	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 45.4–46.6, 3 strains (T_m: Nakase and Komagata 1971d); 53.7, CBS 1752 (T_m: Meyer et al. 1984); 47.5, type strain of *C. mannitofaciens* (BD: Meyer et al. 1984); 46.4, one strain of *Debaryomyces tamarii* (BD: Price et al. 1978); 45.0, type strain; 46.2, type strain of *C. mannitofaciens*; 44.1, type strain of *C. halophila*; 45.4, JCM 5957 and JCM 5974; 46.0, JCM 5958 (T_m: Suzuki et al. 1992).

Origin of the strains studied: Fermenting cucumber brines in U.S.A. (8, including CBS 1731, type strain of *Torulopsis anomala*; CBS 1752, type strain of *C. versatilis*): CBS 5007, sugar, Mauritius; soy mash (3, including CBS 5981, type strain of *C. mannitofaciens*; CBS 4019, type strain of *C. halophila*; CBS 4333, type strain of *Debaryomyces tamarii*).

Type strain: CBS 1752.

Comments: Suzuki et al. (1992) demonstrated the conspecificity of *C. versatilis, C. mannitofaciens, C. halophila, Debaryomyces tamarii* and two strains designated '*C. rhodohalophila*' in studies that included DNA base compositions and reassociations, electrophoretic patterns of enzymes, and proton magnetic resonance (PMR) spectra of cell wall mannans. F.-L. Lee et al. (1992) performed DNA reassociations on these same species and also reported their conspecificity. The proper identification of *D. tamarii* is welcomed since this yeast did not fit well in *Debaryomyces*. Previously, it was noted that it had a higher mol% G+C than the other species in *Debaryomyces* (Nakase and Komagata 1971a, Price et al. 1978) and that it was widely separated from other species of *Debaryomyces* by ribosomal RNA comparisons (Kurtzman and Robnett 1991, Yamada et al. 1991a). Although *D. tamarii* was described as having spherical warty-walled ascospores, no one has been able to confirm this claim. [The mol% G+C value, 53.7%, previously reported for the type strain of *C. versatilis* (Meyer et al. 1984) is most likely an error; possibly a culture mix-up with strains of *C. etchellsii* (52.6%) that have similar CBS numbers.]

64.158. *Candida vinaria* Y. Ohara, Nonomura & Yunome ex M.Th. Smith (1973)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (1.5–2.5)×(3.0–7.0) µm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae consist of branched chains of elongate cells, sometimes with a few ovoidal blastoconidia. Aerobic growth is off-white, shiny, butyrous, smooth and entire.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	l
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	l
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–/l
Melibiose	–	D-Glucitol	–/l
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–/l	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	L-Lysine	+
5-Keto-D-gluconate	–	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	+	10% NaCl/5% glucose	–
L-Arabinitol	–	Starch formation	–
Arbutin	–	Urease	–
Propane 1,2 diol	–	Biotin-free	+
Butane 2,3 diol	–	Pyridoxine-free	+
Nitrite	–	0.1% Cycloheximide	+
Cadaverine	–	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 44.1, type strain (T_m: Nakase and Komagata 1971f).

Origin of the strain studied: CBS 4077, grape must, Japan.

Type strain: CBS 4077.

64.159. *Candida vini* (Vallot ex Desmazières) van Uden & H.R. Buckley ex S.A. Meyer & Ahearn (1983)

Synonyms:

Mycoderma vini Vallot ex Desmazières (1827) [non *Mycoderma vini* Grawitz 1877; nec *Mycoderma vini* de Rossi 1917]
Mycokluyveria vini (Vallot ex Desmaziéres) Ciferri & Redaelli (1947)
Candida vini (Vallot ex Desmazières) van Uden & H.R. Buckley (1970) nom. inval.
Mycoderma cerevisiae Desmazières (1827)
Mycokluyveria cerevisiae (Desmazières) Ciferri & Redaelli (1947)
Saccharomyces mycoderma Reess (1870)
Candida mycoderma (Reess) Lodder & Kreger-van Rij (1952)
Azymocandida mycoderma (Reess) Novák & Zsolt (1961)
Mycoderma gallica Leberle (1909)
Mycoderma vini Vallot ex Desmazières var. *paradoxa* Zimmermann (1938)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are ovoidal, (3.5–5.5)×(4.5–8.0) μm, single, in pairs, and small groups or chains. After one month, islets and an incomplete pellicle may be present.

Growth in yeast nitrogen base+0.5% glucose: After 5 days at 25°C, a pellicle may be present.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae consist of branched chains of elongate cells, sometimes with oval blastoconidia. Aerobic growth is off-white, dull, and creamy, with a slightly granular surface area and a fringed border.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–/l
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–/l
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	v	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	–	Biotin-free	–/l
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.01% Cycloheximide	–
Nitrite	–	Growth at 25°C	+
Cadaverine	+	Growth at 30°C	v
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 34.1, CBS 639 (BD: Meyer et al. 1984); 32.0, CBS 633; 35.4, CBS 639 (T_m: Stenderup et al. 1972); 32.2, CBS 2122; 33.2, CBS 2119 (T_m: Meyer, unpublished data).

Origin of the strains studied: CBS 634, unknown, received from W.B. Bierberg as *Mycoderma cerevisiae*; CBS 639, sour wine; CBS 640, cloudy wine (type of *Mycoderma vini* var. *paradoxus*); CBS 2099, perry; CBS 2109, CBS 2119, wine; CBS 2122, beer.

Type strain: CBS 639 (neotype, van Uden and Buckley 1970, p. 1073).

Comments: Many strains sporulate on 5% Difco malt agar, either alone or more commonly when mixed with a compatible mating type and form hat-shaped ascospores. From preliminary protein electrophoresis studies and DNA comparisons, the strains included in this taxon do not represent a single species (S. Lee and Meyer, unpublished data). Kurtzman and Robnett (unpublished data) found the type strains of *C. vini* and *Pichia fluxuum* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

64.160. *Candida viswanathii* Viswanathan & H.S. Randhawa ex R.S. Sandhu & H.S. Randhawa (1962)

Synonyms:

Candida viswanathii Viswanathan & Randhawa (1959) nom. inval.
Trichosporon lodderae (as *T. lodderi*) Phaff, Mrak & Williams (1952)
Fermentotrichon lodderae (Phaff, Mrak & Williams) Novák & Zsolt (1961)
Candida lodderae (Phaff, Mrak & Williams) S.A. Meyer & Ahearn (1983) [non *Candida lodderae* Kumbhojkar (1981)]

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are subglobose to ovoidal, (3.5–7.0) × (5.0–9.0) μm, and occur singly and in pairs. Some cylindrical cells and pseudohyphae may also be present (Fig. 273).

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudohyphae consist of branched chains of long, cylindrical cells with small verticils of ovoidal blastoconidia; septate hyphae may also be present. Aerobic growth is off-white to chalky-white, dull, dry to powdery, smooth and butyrous or lacy to wrinkled with a mycelial edge.

Fermentation:

Glucose	+	Lactose	–
Galactose	s	Raffinose	–
Sucrose	v	Trehalose	+/l
Maltose	+		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/l
Inulin	–	D-Gluconate	+/l
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–/l	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–/l	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	–

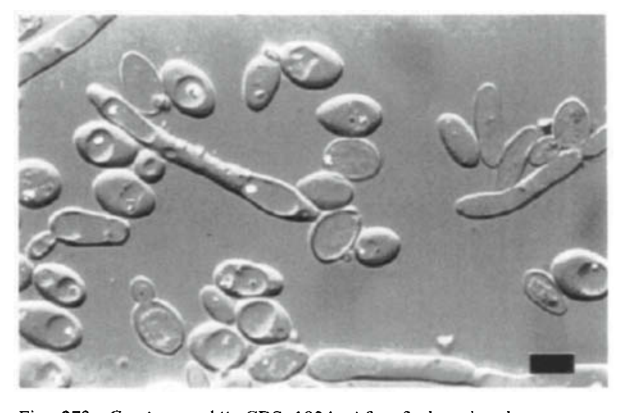


Fig. 273. *C. viswanathii*, CBS 1924. After 3 days in glucose–yeast extract–peptone broth at 25°C. Bar = 5 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	+
D-Glucuronate	–	10% NaCl/5% glucose	+
Xylitol	+/l	Starch formation	–
L-Arabinitol	–/l	Urease	–
Arbutin	+	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	–	Growth at 35°C	+
Cadaverine	+	Growth at 37°C	+
Creatinine	–	Growth at 40°C	v
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G + C: 46.3, type strain, CBS 4024 (T_m: Meyer and Phaff 1972); 45.6, one strain (T_m: Nakase and Komagata 1971g); 45.9, CBS 4024; 45.6, CBS 1924 (T_m: Duncan and Meyer, unpublished data).

Origin of the strains studied: CBS 4024, cerebrospinal fluid, India; CBS 5362, sputum, India; CBS 1924, shrimp (*Peneaus setiferus*), from Gulf of Mexico.

Type strain: CBS 4024.

Comments: Kurtzman and Robnett (1997) found only two nucleotide differences in the comparison of rRNA gene sequences between the type stains of *C. viswanathii* and *C. lodderae*. Mol% G + C contents are similar and first derivative curves of the thermal denaturations are identical (Duncan and Meyer, unpublished data). *Saccharomyces pleomorphus* Lodder (1932) had been considered a synonym of *C. lodderae*. Slight physiological differences exist between these strains. Molecular comparisons of these strains are being done to determine the status of *S. pleomorphus*.

64.161. *Candida wickerhamii* (Capriotti) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Synonym:

Torulopsis wickerhamii Capriotti (1958g)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to subglobose, (3.0–4.5) × (3.0–4.5) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is off-white to cream-colored, smooth, soft, and entire.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	v
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	l	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–/l	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	+
Saccharate	–	D-Glucosamine (N)[1]	–
D-Glucuronate	–	50% Glucose	–
Xylitol	l	10% NaCl/5% glucose	n
L-Arabinitol	–	Starch formation	–
Arbutin	+	Urease	–
Propane 1,2 diol	+	Biotin-free	–
Butane 2,3 diol	l	Pyridoxine-free	+
Nitrite	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 35°C	v
Creatinine	–	Growth at 37°C	–

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 38.1, CBS 2928 (T_m: Meyer et al. 1984).

Origin of the strains studied: CBS 2928, silage, Italy; CBS 6395, insect tunnels in timber, South Africa.

Type strain: CBS 2928.

64.162. *Candida xestobii* Yarrow & S.A. Meyer (1978)

Synonym:

Torulopsis xestobii Jurzitza (1970) nom. nud.

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are globose to ovoidal, (2.2–3.6)×(2.9–4.3) μm, single and in pairs.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae are absent. Aerobic growth is off-white to beige, smooth, glistening, soft and entire.

Fermentation:

Glucose	s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–/l	Ribitol	l
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	l	D-Glucitol	–
Raffinose	l	α-Methyl-D-glucoside	+
Melezitose	–/l	Salicin	–/l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	l
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	l	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	n	D-Glucosamine (N)[1]	n
Saccharate	–	50% Glucose	–
D-Glucuronate	–	10% NaCl/5% glucose	–
Xylitol	+	Starch formation	–
L-Arabinitol	+	Urease	–
Arbutin	–/l	Biotin-free	–
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	–	Growth at 25°C	+
Cadaverine	+	Growth at 30°C	w
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.

Mol% G+C: 47.8, CBS 5975 (T_m: Stenderup et al. 1972); 48.7, CBS 5975 (BD: Meyer et al. 1984).

Origin of the strain studied: CBS 5975, bark beetles (*Xestobium plumbeum*).

Type strain: CBS 5975.

Comments: Kurtzman and Robnett (1997) found *C. fukuyamaensis* and *C. xestobii* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific. Furthermore, the sequence of *Pichia guilliermondii* differed by only one nucleotide, suggesting that it is the teleomorph of these two anamorphic taxa.

64.163. *Candida zeylanoides* (Castellani) Langeron & Guerra (1938)

Synonyms:

Monilia zeylanoides Castellani (1920)
Mycotorula zeylanoides (Castellani) Redaelli & Ciferri (Ciferri and Redaelli 1935)
Parendomyces zeylanoides (Castellani) Dodge (1935)
Pseudomonilia zeylanoides (Castellani) Dodge & Moore (1936)
Azymocandida zeylanoides (Castellani) Novák & Zsolt (1961)
Cryptococcus macroglossiae Castellani (1925)
Monilia macroglossiae Castellani (1925)
Torulopsis macroglossiae (Castellani) Castellani & Jacono (1933)
Mycocandida macroglossiae (Castellani) Redaelli & Ciferri (Ciferri and Redaelli 1935)

Blastodendrion macroglossiae (Castellani) Langeron & Guerra (1935)
Parendomyces macroglossiae (Castellani) Dodge (1935)
Monilia zeylanoides Castellani var. *macroglossiae* Castellani (1937a)
Cryptococcus uvae Pollacci & Nannizzi (Motta 1926)
Monilia uvae (Pollacci & Nannizzi) Vuillemin (1931)
Torulopsis uvae (Pollacci & Nannizzi) Lodder (1934)
Syringospora uvae (Pollacci & Nannizzi) Dodge (1935)
Blastodendrion canis von Szilvinyi (1934)
Monilia parazeylanoides Castellani (1937a)
Pichia dubia Dietrichson (1954)
Trichosporon piscium Siepmann (Siepmann and Höhnk 1962)
Candida iberica C. Ramírez & C. González (1972)

Growth in glucose–yeast extract–peptone broth: After 3 days at 25°C, the cells are oval to elongate, (2.5–7.0)×(5.0–10.0) μm, single, in pairs and short chains. After one month a pellicle may be present.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, pseudohyphae consist of branched chains of cylindrical cells, often with verticils of oval blastoconidia. Aerobic growth is off-white to cream-colored, shiny, smooth to wrinkled and entire to fringed.

Fermentation:

Glucose	–/s	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–/s
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+/l
Galactose	–/l	Methanol	–
L-Sorbose	+/l	Ethanol	v
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–/l	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+/l
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–/l	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	v
5-Keto-D-gluconate	+	D-Glucosamine (N)[1]	–
Saccharate	n	50% Glucose	v
D-Glucuronate	–	10% NaCl/5% glucose	v
Xylitol	–	Starch formation	–
L-Arabinitol	–	Urease	–
Arbutin	v	Biotin-free	v
Propane 1,2 diol	–	Pyridoxine-free	+
Butane 2,3 diol	–	0.1% Cycloheximide	+
Nitrite	v	Growth at 30°C	+
Cadaverine	v	Growth at 35°C	v
Creatinine	–	Growth at 37°C	w/–
L-Lysine	v		

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 57.6, CBS 619 (T_m: Stenderup and Bak 1968); 54.5, 2 strains (T_m: Nakase and Komagata 1968a); 55.9, ATCC 7351 (CBS 619); 54.7, AJ 4741 (T_m: Nakase and Komagata 1971f).

Origin of the strains studied: CBS 619, blastomycotic macroglossia, Sri Lanka; CBS 641, CBS 2328, chilled beef, Australia; CBS 947, throat; CBS 1922, unknown; CBS 2000, sputum, Norway; CBS 2326, dog's skin; CBS 2329, probably human; CBS 4909, sea water; CBS 5122, skin; CBS 5262, dolphin's skin; CBS 5446, 5447, salami, Yugoslavia; CBS 5718, red clover, Canada; CBS 6134, 6135, feces, Finland; 6165, probably human; CBS 6391, 6408, 6409, 6410, 6411, sausages, Spain.

Type strain: CBS 619 (neotype, Diddens and Lodder 1942, p. 304).

Comments: Kurtzman and Robnett (1997) found *C. krissii* and *C. zeylanoides* to have identical sequences in a 600-nucleotide region at the 5′ end of large subunit (26S) rDNA, indicating the two taxa to be conspecific.

Comments on the genus

Viljoen and Kock (1989b) presented a chronological account of the history of the genus *Candida* Berkhout with the rationale of various investigators for the changes that took place until the delimitation of the genus to anamorphs with only ascomycetous affinity. Several investigators (von Arx and Weijman 1979, Moore 1980, Golubev 1981, Weijman and Rodrigues de Miranda 1988) reclassified the basidiomycetous *Candida* species and transferred them to other genera. Van der Walt (1987) made the necessary changes to separate the ascomycetous and basidiomycetous anamorphic yeasts into appropriate families. He reinstated the family name Candidaceae for the ascomycetous anamorphic yeasts (to which *Candida* was assigned) and restricted the family Cryptococcaceae to basidiomycetous anamorphs and the family Sporobolomycetaceae to anamorphs termed ustomycetous. Weijman and Rodrigues de Miranda (1988) compared members of the genus *Candida* on the basis of carbohydrate patterns of intact whole cell hydrolyzates. They found three groups. The first group represented ascomycetous yeasts where mannose dominated, and rhamnose, fucose and xylose were absent. The basidiomycetous yeasts were distributed in the other two groups; one characterized by the presence of xylose and low mannose and the other by the presence of fucose and/or rhamnose with significant amounts of mannose. Then, Weijman et al. (1988) redefined the genus *Candida,* emended *Cryptococcus* and *Rhodotorula,* and transferred the basidiomycetous *'Candida'* species to the appropriate emended genera.

Although the species with a basidiomycetous affinity have been removed from the genus, *Candida* still remains heterogeneous. The mol% G+C ranges from 30–62.7%, Co-Q 6, 7, 8 and 9 are present, fermentation may be absent to strong, cells vary in shape and size, and assimilatory properties vary. *Candida* species that were found to sporulate have teleomorphic states in eleven different genera. The taxonomic boundaries of *Candida* still remain

broad and essentially any asexual yeast that does not fit the criteria of some other genus will find its way into *Candida*. In particular, *Candida* acts as a depository for all the asexual yeasts with ascomycetous affinity except those with: (a) acetic acid production (*Brettanomyces*), (b) bipolar budding on a broad base (*Kloeckera* and *Schizoblastosporion*), (c) triangular cells (*Trigonopsis*), (d) blastoconidia formed on sympodulae (*Sympodiomyces*) or on pedicils or denticles (*Blastobotrys*), (e) dichotomously branched terminal pseudohyphal cells (*Botryozyma*), (f) needle-shaped terminal conidia (*Aciculoconidium*), and (g) arthroconidia (*Arxula, Geotrichum*), (h) carotenoid pigments (*Lalaria, Oosporidium, Saitoella*), (i) extracellular starch-like compounds (*Myxozyma*). In some instances, *Candida* is a temporary repository for a species until ascosporulation is observed and it can be placed in a teleomorphic genus. Some species are represented by only a single strain. A number of *Candida* species have been found to sporulate, either independently or when strains are mixed and mating types are revealed. The list of *Candida* species presented here includes some species that have teleomorphic counterparts. Some are often isolated as single mating types, such as *C. guilliermondii* and *C. lusitaniae,* or others are well-known species whose relationship to the teleomorph was established by DNA reassociation (*C. krusei, C. sorbosa*). In addition, there are other species that were at one time included in *Candida,* but because they were found to sporulate are not listed in *Candida,* but in the appropriate ascomycetous genera. These species (anamorph/teleomorph) are cross-referenced in the index. When sporulation is elusive, molecular techniques can be used to resolve relationships. DNA–DNA reassociation and ribosomal RNA gene sequencing are techniques that have been employed to determine anamorphic/teleomorphic relationships, as well as conspecificity of *Candida* species and proper identity of strains within a species. These techniques have been particularly useful in resolving the correct status of a number of *Candida* species.

Some species that were published while this chapter was being prepared are not included, namely: *C. fragi* Suzuki, Nakase & Kukazawa (1991), *C. pseudoglaebosa* Suzuki & Nakase (1993), *C. sojae* Nakase, Suzuki, Takashima, Miyakawa, Kagaya, Fukazawa & Komagata (1994b), *C. stellimalicola* Suzuki, Nakase & Komagata (1994), *C. cellulolytica* and *C. fukuyamaensis* Nakase, Suzuki, Takashima, Hamamoto, Hatano & Fukui (1994c), and *C. dubliniensis* Sullivan, Westerneng, Haynes, Bennett & Coleman (1995). *C. fragi* has physiological properties similar to *C. sake, C. oleophila* and *C. natalensis,* but it is distinct from them on the basis of DNA relatedness, electrophoretic enzyme patterns and proton magnetic resonance (PMR) spectra of the cell wall mannans. *C. sojae,* a species isolated from an extraction process of water-soluble substances of defatted soybean flakes, has some similarity to *C. albicans* and *C. tropicalis.* However, it is distinguished from these species on mol% G+C, electrophoretic enzyme patterns and the PMR spectra of cell wall mannans. *C. stellimalicola,* a species isolated from Ma-Fueng (star apple) in Thailand, shows some similarity to *C. diversa, C. silvae* and *C. karawaiewii.* It was distinguished from these species by DNA reassociation, proton magnetic resonance spectra of the cell wall mannans and serological characteristics of cell surface antigens. *C. cellulolytica* and *C. fukuyamaensis* are both cellulolytic yeasts isolated from natural habitats by enrichment techniques. The latter species has some similarities with *C. famata, C. guilliermondii* and *C. salmanticensis,* but can be differentiated from them on various criteria. *C. dubliniensis* was recently described for a number of strains isolated from HIV-infected patients and uninfected persons. It has characteristics that resemble *C. albicans.* It is germ-tube positive and produces chlamydospores in abundance. Its physiological profile is nearly identical to that of *C. albicans.* Sullivan et al. (1995) employed several molecular biological techniques to compare strains of this species with *C. albicans* strains. *C. albicans* serotype A and serotype B strains and the type strain and one other strain of *C. stellatoidea* were included in the study. The results showed that the strains of *C. dubliniensis* were distinct from the *C. albicans* strains. See the comments section of *C. albicans* for more information.

Species of uncertain affiliation: *C. santamariae* var. *membranifaciens* and *C. rignihuensis* were previously included in *C. santamariae* and *C. oleophila,* respectively. *C. molischiana* was previously considered the anamorph of *Pichia capsulata.* However, this relationship is not supported by ribosomal RNA gene sequence comparisons (Kurtzman, personal communication). Also, the status of *Saccharomyces pleomorphus,* a strain previously considered a synonym of *C. lodderae,* is questioned because the type strain of *C. lodderae* was found to be conspecific with *C. viswanathii.*

Species complexes: Some species are heterogeneous. They include unrelated strains whose relationships have not been resolved. *C. sake* and *C. oleophila* are excellent examples. *C. intermedia, C. rugosa, C. tenuis* and *C. zeylanoides* show much variability and are possibly heterogeneous. Others, such as *C. parapsilosis* and *C. haemulonii* have been shown to have two or more distinct genomic types (refer to the comments section of the species descriptions for more information).

65. *Geotrichum* Link:Fries

G.S. de Hoog, M.Th. Smith and E. Guého

Diagnosis of the genus

Colonies are white, farinose or hairy, usually dry, and consist of true hyphae which disarticulate into rectangular arthroconidia. Additionally, mostly sympodially produced conidia may be present. Budding is absent. Chlamydospores and endoconidia may be present. Septa have micropores.

Fermentation is mostly absent. Nitrate is not assimilated. Extracellular starch is not produced. Diazonium blue B reaction is negative.

Teleomorph genera: *Dipodascus, Galactomyces.*

Type species

Geotrichum candidum Link:Fries

Species accepted

1. *Geotrichum candidum* Link:Fries (1832): see *Galactomyces geotrichum*, p. 210
2. *Geotrichum capitatum* (Diddens & Lodder) von Arx (1977): see *Dipodascus capitatus,* p. 186
3. *Geotrichum citri-aurantii* (Ferraris) E.E. Butler (1988): see *Galactomyces citri-aurantii*, p. 209
4. *Geotrichum clavatum* de Hoog, M.Th. Smith & Guého (1986)
5. *Geotrichum decipiens* (L. Tulasne & R. Tulasne) W. Gams (1983): see *Dipodascus armillariae,* p. 184
6. *Geotrichum fermentans* (Diddens & Lodder) von Arx (1977)
7. *Geotrichum fragrans* (Berkhout) Morenz ex Morenz (1964)
8. *Geotrichum ingens* (van der Walt & van Kerken) de Hoog, M.Th. Smith & Guého (1997): see *Dipodascus ingens,* p. 188
9. *Geotrichum klebahnii* (Stautz) Morenz (1964)
10. *Geotrichum ludwigii* (E.C. Hansen) Fang, Cheng & Chu (1966): see *Dipodascus magnusii,* p. 189
11. *Geotrichum sericeum* (Stautz) de Hoog, M.Th. Smith & Guého (1986): see *Dipodascus ovetensis,* p. 190

Physiological and morphological key to species of *Geotrichum, Dipodascus* and *Galactomyces*

See Table 62.

1.	a D-Xylose assimilated → 2			
	b D-Xylose not assimilated → 11			
2(1).	a Cellobiose assimilated → 3			
	b Cellobiose not assimilated → 6			
3(2).	a Salicin assimilated → 4			
	b Salicin not assimilated → 5			
4(3).	a D-Glucitol assimilated	....	*Geotrichum fermentans*:	p. 576
	b D-Glucitol not assimilated	....	*Dipodascus spicifer*:	p. 191
5(3).	a L-Sorbose assimilated	....	*Dipodascus macrosporus*:	p. 189
	b L-Sorbose not assimilated	....	*Dipodascus armillariae*:	p. 184
6(2).	a Maltose assimilated	....	*Dipodascus geniculatus*:	p. 187
	b Maltose not assimilated → 7			
7(6).	a Growth in vitamin-free medium	....	*Galactomyces geotrichum*:	p. 210
			Geotrichum klebahnii:	p. 578
	b Absence of growth in vitamin-free medium → 8			
8(7).	a Mannitol assimilated → 9			
	b Mannitol not assimilated	....	*Galactomyces reessii*:	p. 212
9(8).	a Growth at 37°C	....	*Dipodascus australiensis*:	p. 185
	b Absence of growth at 37°C → 10			
10(9).	a Ribitol assimilated	....	*Dipodascus aggregatus*:	p. 182
			Galactomyces citri-aurantii:	p. 209
	b Ribitol not assimilated	....	*Dipodascus albidus*:	p. 183
11(1).	a Sucrose assimilated	....	*Dipodascus magnusii*:	p. 189
	b Sucrose not assimilated → 12			
12(11).	a Cellobiose assimilated	....	*Geotrichum clavatum*:	p. 575
	b Cellobiose not assimilated → 13			

13(12). a Culture odor strongly fruity .... *Geotrichum fragrans*: p. 577
b Culture odor insignificant or faintly fruity → 14
14(13). a D-Glucitol assimilated → 15
b D-Glucitol not assimilated → 16
15(14). a Citrate assimilated .... *Dipodascus tetrasperma*: p. 192
b Citrate not assimilated .... *Dipodascus ambrosiae*: p. 184
16(14). a Hyphae markedly constricted at septa .... *Dipodascus ingens*: p. 188
b Hyphae not markedly constricted at septa → 17
17(16). a Growth at 37°C .... *Dipodascus capitatus*: p. 186
b Absence of growth at 37°C .... *Dipodascus ovetensis*: p. 190

Table 62
Key characters of species in the genera *Geotrichum*, *Dipodascus* and *Galactomyces*

Species	Assimilation[a]										Growth[b]		Fruity odor[c]	Septal constrict[d]
	Sor	Suc	Mal	Cel	Xyl	Rbl	Mtl	Glt	Sal	Cit	Vfree	37°C		
Geotrichum clavatum	+	–	–	+	–	–	–	–	+	+	–	+	–	–
G. fermentans	+	–	–	+	+	v	+	+	+/w	+	+	v	–	–
G. fragrans	+	–	–	–	–	–	v	v	–	v	–	v	+	–
G. klebahnii	+	–	–	–	+	v	+	+	–	v	+	–	–	–
Dipodascus aggregatus	+	–	–	–	+	+	+	+	–	+	–	–	–	–
D. albidus	+	–	–	–	+	–	+	+	–	–	–	–	–	–
D. ambrosiae	v	–	–	–	–	v	+	+	–	–	–	–	–	–
D. armillariae	–	–	–	+	+	–	+	v	–	v	+	–	–	–
D. australiensis	+	–	–	–	+	v	+	+	–	–	–	+	–	–
D. capitatus	v	–	–	–	–	–	–	–	–	v	–	+	–	–
D. geniculatus	+	–	+	–	+	–	+	+	–	+	–	–	–	–
D. ingens	v	–	–	–	–	–	–	–	–	–	+	v	–	+
D. macrosporus	+	–	–	+	+	v	+	+	–	+	+	–	–	–
D. magnusii	+	+	–	–	–	–	+	+	–	–	–	–	–	–
D. ovetensis	v	–	–	–	–	–	–	–	–	–	v	–	–	–
D. spicifer	+	–	–	+	+	–	–	–	+	+	–	+	–	–
D. tetrasperma	+	–	–	–	–	–	–	+	–	+	–	+	–	–
Galactomyces citri-aurantii	+	–	–	–	+	+	+	+	–	+	–	–	–	–
G. geotrichum	+	–	–	–	+	v	v	+	–	v	+	v	–	–
G. reessii	+	–	–	–	+	–	–	+	–	v	–	v	–	–

[a] Abbreviations: Sor, L-sorbose; Suc, sucrose; Mal, maltose; Cel, cellobiose; Xyl, D-xylose; Rbl, ribitol; Mtl, D-mannitol; Glt, D-glucitol; Sal, salicin; Cit, citrate.
[b] Abbreviations: Vfree, in vitamin-free medium; 37°C, at 37°C.
[c] Culture produces a strong fruity odor.
[d] Hyphae are markedly constricted at the septa.

Systematic discussion of the species

65.1. *Geotrichum candidum* Link:Fries (1832)

See under teleomorph *Galactomyces geotrichum*: p. 210.

65.2. *Geotrichum capitatum* (Diddens & Lodder) von Arx (1977a)

See under teleomorph *Dipodascus capitatus*: p. 186.

65.3. *Geotrichum citri-aurantii* (Ferraris) E.E. Butler (Butler et al. 1988)

See under teleomorph *Galactomyces citri-aurantii*: p. 209.

65.4. *Geotrichum clavatum* de Hoog, M.Th. Smith & Guého (1986)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 15–17 mm in diameter, glassy-white, flat, smooth and tough. Expanding hyphae are in loose bundles and break up into rectangular cells of variable size, (2.8–4.0)×(6–20) μm. Terminal parts of hyphae may swell and become thick-walled; the widest cell at the apex measures 8×11 μm (Fig. 274).

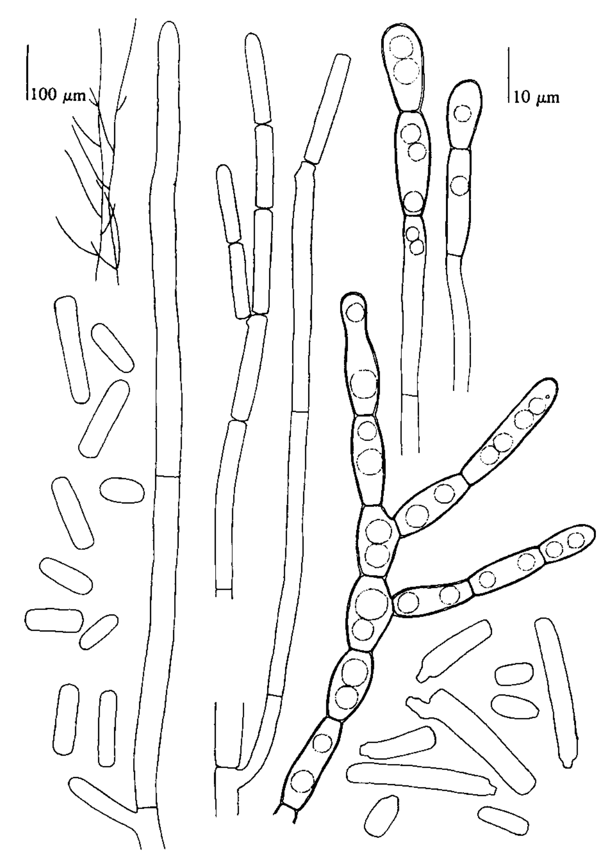


Fig. 274. *G. clavatum*, CBS 576.82, 425.71. MEYA, 22°C. Branching pattern arthroconidia and swollen terminal cells.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	n	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	+
Arbutin	+		

Co-Q: Not determined.

Mol% G+C: 31.1–33.2, CBS 425.71, CBS 576.82 (T_m: de Hoog et al. 1986).

Origin of the strains studied: CBS 425.71, from human lung tissue, Ahearn, Atlanta, U.S.A.; CBS 576.82 (CBS 2465), from human patient with asthma, Orie, Netherlands.

Type strain: CBS 425.71.

Comments: This is one of the arthroconidial opportunists involved in human systemic mycoses, particularly in connection with pulmonary disorders. For a discussion on emerging systemic opportunists, see under *Dipodascus capitatus*.

65.5. *Geotrichum decipiens* (L. Tulasne & R. Tulasne) W. Gams (1983)

See under teleomorph *Dipodascus armillariae*: p. 184.

65.6. *Geotrichum fermentans* (Diddens & Lodder) von Arx (1977a)

Synonyms:

Trichosporon fermentans Diddens & Lodder (1942)
Fermentotrichon fermentans (Diddens & Lodder) Novák & Zsolt (1961)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 35–40 mm in diameter, whitish, moist and somewhat raised, with straight and sharp margins that later become fimbriate margins. Expanding hyphae are straight, initially remotely septate, later every 20–60 μm; main branches are 5–7 μm wide, lateral branches are 2.5–4.0 μm wide, and disarticulate into arthroconidia, (2.8–4.0) × (8–30) μm. Hyphae that form later often remain intact and develop short grape-like branches composed of ellipsoidal cells (Fig. 275).

Fermentation:

Glucose	+	Lactose	–
Galactose	+	Raffinose	–
Sucrose	–	Trehalose	n
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+/w
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	v
Arbutin	+		

Co-Q: Not determined.

Mol% G+C: 42, CBS 439.83 (T_m: Guého 1979), 46.1–46.2, CBS 409.34, CBS 439.83 (BD: Guého et al. 1984).

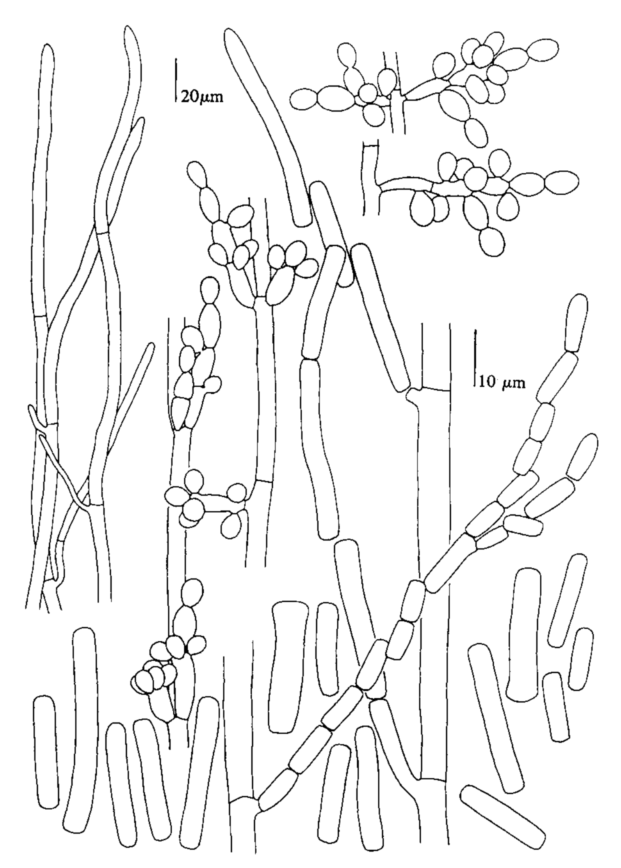


Fig. 275. *G. fermentans*, CBS 2529. MEYA, 22°C. Expanding hyphae, blasto- and arthroconidia.

Origin of the strains studied: CBS 439.83 (CBS 2529, NRRL Y-1492, ATCC 10675) from wood pulp, Rennerfelt, Sweden; from wood pulp mill (1); from tanned sheep skin (1).

Type strain: CBS 439.83.

Comments: One of the main characteristics of this species is the relatively long and narrow conidia which do not inflate after detachment, and the local, grape-like lateral branchlets.

65.7. *Geotrichum fragrans* (Berkhout) Morenz ex Morenz (1964)

Synonyms:

Oospora fragrans Berkhout (1923)
Cylindrium fragrans (Berkhout) Burns (1933)
Endomyces lactis (Fresenius) Windisch var. *fragrans* (Berkhout) Windisch (1951)
Oidium suaveolens Krzemecki (1913)
Oospora gigas Smit & Meyers (1928)
Geotrichum magnum Saëz (1968a)
Geotrichum fici S. Goto, Yamakawa & Yokotsuka (1975b)
Geotrichum rectangulatum S. Goto, Yamakawa & Yokotsuka (1975b)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 25–30 mm in diameter, whitish, tough, hispid with creeping hyphal fascicles, and produce a strong fruity odor. Expanding hyphae are straight and stiff, unilaterally branched at acute angles, main branches are 7–12 μm wide, lateral branches are 4–7 μm wide, and mostly without secondary branching. Hyphae disarticulate into rectangular arthroconidia, (4–7)×(10–18) μm, often with some annellations at one or both ends, and they are intermingled with smaller conidia from lateral branches. Endoconidia, chlamydospores and rhizoids may be present (Fig. 276).

Fermentation:

Glucose	+	Lactose	–
Galactose	v	Raffinose	–
Sucrose	–	Trehalose	n
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	n	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	v
Arbutin	–		

Co-Q: Not determined.
Mol% G+C: 42.2–44.3, 5 strains (T_m: de Hoog et al. 1986).

Origin of the strains studied: CBS 140.25, type strain of *Oospora gigas*, from fruit juice of *Arenga*, Smit; CBS 152.25, type strain of *Oidium suaveolens*, from water, Krzemecki; CBS 126.76, type strain of *Geotrichum rectangulatum*, from oily debris, Goto, Japan; CBS 127.76, type strain of *G. fici*, from fig (*Ficus* sp.) Goto, Japan; palm wine (1); mash of maize (1); from human pulmonary infections (2).

Type strain: CBS 152.25.

Comments: *Oidium suaveolens* is the oldest epithet available for this taxon, but the combination *Geotrichum suaveolens* had already been made for a species now known as *Moniliella suaveolens*. The well-known name *Geotrichum fragrans* can therefore be maintained. De Hoog et al. (1986) found that several strains of the species show a very low degree of DNA/DNA reassociation, which would point to taxonomic heterogeneity. Thus far no usable key features are available to distinguish these sibling taxa. The species is regularly found in nutrient-rich, liquid substrates, particularly in industrial waste water.

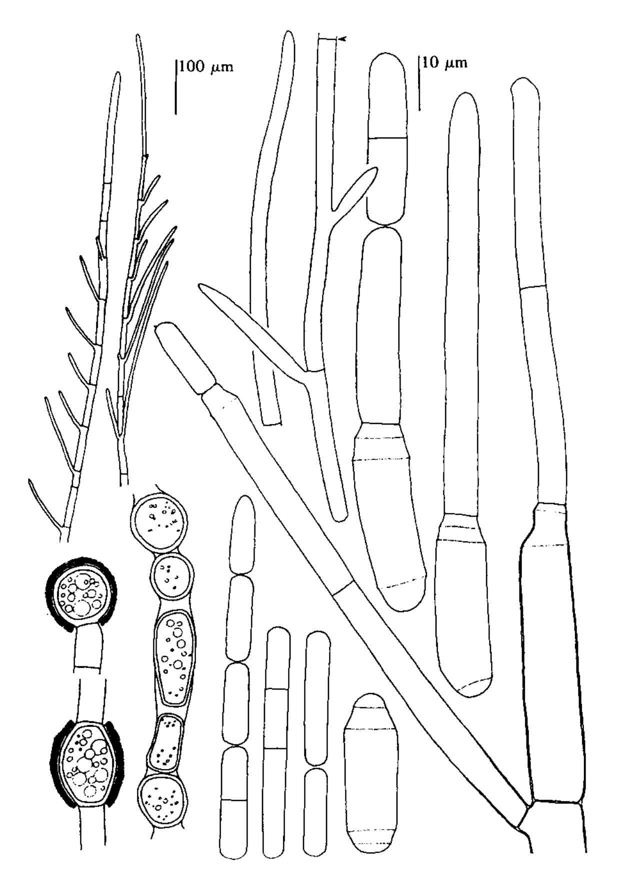


Fig. 276. *G. fragrans*, various CBS strains. MEYA, 22°C. Expanding hyphae with penicillate arthroconidia and conidia with annellated zones.

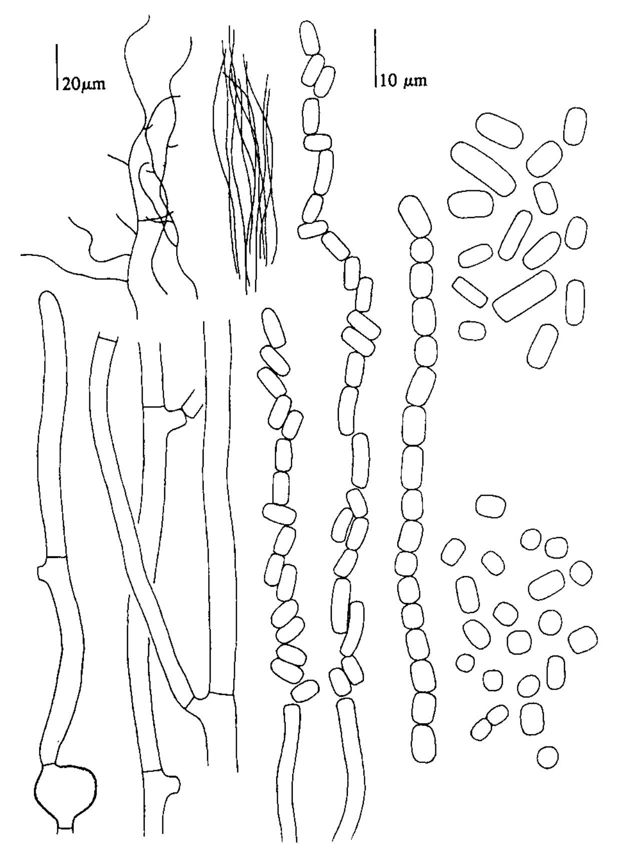


Fig. 277. *G. klebahnii*, various CBS strains. MEYA, 22°C. Expanding hyphae and arthroconidia.

65.8. *Geotrichum ingens* (van der Walt & van Kerken) de Hoog, M.Th. Smith & Guého (1997a)

See under teleomorph *Dipodascus ingens*: p. 188.

65.9. *Geotrichum klebahnii* (Stautz) Morenz (1964)

Synonyms:

Trichosporon klebahnii Stautz (1931)
Endomyces lactis (Fresenius) Windisch var. *klebahnii* (Stautz) Windisch (1951)
Trichosporon penicillatum do Carmo-Sousa (1965)
Geotrichum penicillatum (do Carmo-Sousa) von Arx (1977a)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 25–30 mm in diameter, glassy-white, moist, somewhat raised and slightly veined, with margins that are fimbriate, and finally hairy. Expanding hyphae are septate every 40–80 µm, 3.5–5.0 µm in width, with slight differentiation of main and lateral branches. Hyphae locally disarticulate into cubic arthroconidia measuring (2.8–3.2)×(4.5–9.0) µm (Fig. 277).

Fermentation:

Glucose	+	Lactose	–
Galactose	w/–	Raffinose	–
Sucrose	–	Trehalose	n
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 37°C	–
Arbutin	–		

Co-Q: Not determined.

Mol% G+C: 40.1–40.7, CBS 179.30, CBS 627.74 (BD: Guého et al. 1984).

Origin of the strains studied: CBS 179.30, from slime flux of yew (*Taxus baccata*), Stautz; CBS 627.74 (CBS 5586, ATCC 18019), type strain of *T. penicillatum*, from

slime flux of elm (*Ulmus* sp.), Phaff; from wood pulp (1); from sewage filter (1).

Type strain: CBS 179.30.

Comments: The occurrence of this species in slime flux of trees is striking. It is probably selected by high concentrations of sugars. It was also reported from corn pulp (Glanser and Ban 1983) and sugar cane bagasse (Pou et al. 1985). *G. klebahnii* is close to *G. clavatum,* but differs in growth with D-xylose. The ecological preferences of the two species are markedly different; *G. clavatum* is a human pathogen. An additional species that can be found in tree exudates is the *Geotrichum* anamorph of *Dipodascus australiensis.* The latter species is non-fermentative and cannot grow without vitamins. *G. ludwigii* from oak slime flux has a much larger micromorphology.

65.10. *Geotrichum ludwigii* (E.C. Hansen) Fang, Cheng & Chu (1966)

See under teleomorph *Dipodascus magnusii*: p. 189.

65.11. *Geotrichum sericeum* (Stautz) de Hoog, M.Th. Smith & Guého (1986)

See under teleomorph *Dipodascus ovetensis*: p. 190.

66. *Kloeckera* Janke

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is by bipolar budding in basipetal succession on a narrow base. Cells are apiculate, ovoidal to long-ovoidal or elongate. Pseudohyphae are absent, or if present, rarely well-developed. Ascospores are not produced.

Glucose is fermented. Nitrate is not assimilated. Inositol and pantothenate are required for growth. Acetic acid is not produced. Diazonium blue B reaction is negative.

The teleomorphic genus is *Hanseniaspora*.

Type species

Kloeckera apiculata (Reess emend. Klöcker) Janke

Species accepted

1. *Kloeckera africana* (Klöcker) Janke (1928): see *Hanseniaspora vineae,* p. 219
2. *Kloeckera apiculata* (Reess emend. Klöcker) Janke (1928): see *Hanseniaspora uvarum,* p. 217
3. *Kloeckera apis* Lavie ex M.Th. Smith, Simione & S.A. Meyer (1977): see *Hanseniaspora guilliermondii,* p. 215
4. *Kloeckera corticis* (Klöcker) Janke (1928): see *Hanseniaspora osmophila,* p. 216
5. *Kloeckera japonica* Saito & Ohtani (1931): see *Hanseniaspora valbyensis,* p. 218
6. *Kloeckera javanica* (Klöcker) Janke (1928): see *Hanseniaspora occidentalis,* p. 215
7. *Kloeckera lindneri* (Klöcker) Janke (1928)

Key to species

See Table 63.

1.	a	Growth at 37°C	*K. apis*:	p. 581
	b	Growth absent at 37°C → 2		
2(1).	a	Sucrose fermented	*K. javanica*:	p. 581
	b	Sucrose not fermented → 3		
3(2).	a	2-Keto-D-gluconate assimilated	*K. apiculata*:	p. 580
	b	2-Keto-D-gluconate not assimilated → 4		
4(3).	a	Growth absent with 0.01% cycloheximide → 5		
	b	Growth present with 0.01% cycloheximide → 6		
5(4).	a	Growth at 34°C	*K. africana*:	p. 580
	b	Growth absent at 34°C	*K. corticis*:	p. 581
6(4).	a	Growth at 30°C	*K. lindneri*:	p. 581
	b	Growth absent at 30°C	*K. japonica*:	p. 581

Table 63
Key characters of species in the genus *Kloeckera*

Species	Sucrose fermentation	Assimilation of 2-keto-D-gluconate	Growth			
			With 0.01% cycloheximide	At 30°C	At 34°C	At 37°C
Kloeckera africana	–	–	–	+	+	–
K. apiculata	–	+	+	+	n	–
K. apis	–	+	+	+	+	+
K. corticus	–	–	–	+	–	–
K. japonica	–	–	+	–	–	–
K. javanica	+	–	–	+	n	–
K. lindneri	–	–	+	+	n	–

Systematic discussion of the species

66.1. *Kloeckera africana* (Klöcker) Janke (1928)

See teleomorph *Hanseniaspora vineae* van der Walt & Tscheuschner: p. 219.

66.2. *Kloeckera apiculata* (Reess emend. Klöcker) Janke (1928)

See teleomorph *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak & Phaff: p. 217.

66.3. *Kloeckera apis* Lavie ex M.Th. Smith, Simione & S.A. Meyer (1977)

See teleomorph *Hanseniaspora guilliermondii* Pijper: p. 215.

66.4. *Kloeckera corticis* (Klöcker) Janke (1928)

See teleomorph *Hanseniaspora osmophila* (Niehaus) Shehata, Mrak & Phaff: p. 216.

66.5. *Kloeckera japonica* Saito & Ohtani (1931)

See teleomorph *Hanseniaspora valbyensis* Klöcker: p. 218.

66.6. *Kloeckera javanica* (Klöcker) Janke (1928)

See teleomorph *Hanseniaspora occidentalis* M.Th. Smith: p. 215.

66.7. *Kloeckera lindneri* (Klöcker) Janke (1928)

Synonym:
Pseudosaccharomyces lindneri Klöcker (1912b)

Growth in glucose–yeast extract–peptone water: After 2 days at 25°C, cells are apiculate, ovoidal or elongate, (2.0–4.5)×(4.0–9.5) µm, single or in pairs. Reproduction is by bipolar budding. Sediment is present. After one month a thin ring is formed.

Growth on glucose–yeast extract–peptone agar: After one month the streak culture is white to cream-colored, and smooth and glossy; in cross section there is a raised center and a flat periphery.

Dalmau plate cultures on potato agar: Pseudomycelium is absent.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Raffinose	–
Sucrose	–	Trehalose	–
Maltose	–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	0.01% Cycloheximide	+
Starch formation	–	Growth at 34°C	–

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: CBS 285, soil, Java, Kufferath.

Type strain: CBS 285, isolated by Kufferath.

Comments: *Kloeckera lindneri* shows poor growth in the media used for growth tests. However, growth is stimulated by the addition of yeast autolyzate to the media.

Comments on the genus

In 1978, Meyer et al. correlated the six anamorphic *Kloeckera* species with six teleomorphic species in *Hanseniaspora* from comparisons of DNA relatedness. The status of *K. lindneri* was not settled by these authors since the type strain was not available during their study. However, from the physiology, this species does not appear to be a synonym of any of the known species of *Hanseniaspora.* This possibility is supported by the rDNA sequence data obtained by Boekhout et al. (1994) in a study on the phylogenetic relationships of *Hanseniaspora, Kloeckera* and other genera. These authors suggested that *K. lindneri* may represent an anamorph of a *Hanseniaspora* species closely related to *H. uvarum, H. guilliermondii* and *H. valbyensis.* Yamada et al. (1993) determined the phylogenetic relationships of species of the apiculate yeast genera *Wickerhamia* and *Kloeckera* on the basis of the partial sequences of 18S and 26S ribosomal RNAs. The single species *Wickerhamia fluorescens* was found to be phylogenetically separate from species of *Kloeckera* and *Hanseniaspora.*

67. *Lalaria* R.T. Moore

R.T. Moore

Introduction

The order Taphrinales is dimorphic: the teleomorphic phase is mycelial and obligately parasitic on a variety of plant hosts while the anamorphic phase is saprobic and yeastlike. The holomorphic biosystematics of these fungi – the development of biological species concepts for them – has been impeded by the lack of anamorph names for their yeast phases. Only three of the seven genera are available from major commercial culture collections: *Protomyces, Protomycopsis* and *Taphrina.* Most of the *Taphrina* species were isolated by Mix in the course of his many years of study of the genus. Mix (1954) examined 74 isolates, representing 26 species and 55 host forms for their ability to use 32 different carbon compounds. All isolates gave a common response to 12 compounds. All but the two isolates of *T. polystichi* used xylose and all but the isolates of *T. polystichi* and the isolate of *T. nana* used succinic acid. The isolates exhibited various responses to 18 other compounds. In an earlier paper, Mix (1953) examined the ability of these isolates to utilize nitrogen compounds and found that they could all utilize urea and asparagine. Little serious attention has been given subsequently to the systematic characterization of these yeast forms, and to providing them with standard descriptions. Despite their economic importance they have been largely ignored by plant pathology and although Mix (1935) said that the yeast-phase should be common on plant surfaces and probably in the soil, there is no mention of these species in this literature (e.g., do Carmo-Sousa 1969a, Last and Price 1969, Preece and Dickinson 1971, Phaff and Starmer 1987).

Moore (1990), recognizing that part of the problem was the lack of a name to identify the anamorph phase, proposed the name *Lalaria* as an *omnium gatherum* for all the form-species in the order. He recast Mix's data, originally set out in paragraph style, in tabular form. Despite the datedness and, by current standards, incompleteness of these data, they are still the only comprehensive information that we have on these forms. Consequently, the recognized species of *Lalaria* are, at present, all anamorphs of species of *Taphrina.* Although they are defined strictly on the basis of small differences on a limited number of physiological tests, they do parallel the respective teleomorphs, indicating that they probably are taxonomically distinct.

Diagnosis of the genus

Colonies are pinkish or sometimes yellowish due to carotenoid pigments; budding is holoblastic. The species are diazonium blue B and urease negative; species assimilate glucose, maltose, sucrose, trehalose, melezitose, inulin, urea and asparagine, but not lactose, L-rhamnose, erythritol, inositol, or ammonium acetate. They do not ferment glucose, form extracellular starch-like compounds and do not require thiamine. They have Co-Q 10, and a temperature maximum of 28°C, though many isolates grow poorly above about 20°C.

Type species

Lalaria populina R.T. Moore

Species accepted

1. *Lalaria americana* R.T. Moore (1990)
   Teleomorph: *Taphrina americana* Mix
   Host: *Betula fontinalis* Sargent
   Origin: Mix (1954), no. 1 (CBS 331.55)
2. *Lalaria caerulescens* R.T. Moore (1990)
   Teleomorph: *Taphrina caerulescens* (Montagne & Desmazières) Tulasne
   Host: *Quercus alba* L.
   Origin: Mix (1954), no. 5 (CBS 351.35)
3. *Lalaria carnea* R.T. Moore (1990)
   Teleomorph: *Taphrina carnea* Johanson
   Host: *Betula intermedia* Thomas
   Origin: Mix (1954), no. 21 (CBS 332.55, MUCL 30956)
4. *Lalaria cerasi* R.T. Moore (1990)
   Teleomorph: *Taphrina cerasi* (Fuckel) Sadebeck
   Host: *Prunus avium* L.
   Origin: Mix (1954), nos. 22, 23, 24 (CBS 275.28)

5. *Lalaria coccinea* R.T. Moore (1990)
   Teleomorph: *Taphrina caerulescens* (Montagne & Desmazières) Tulasne
   Host: *Quercus coccinea* Münch
   Origin: Mix (1954), no. 7 (CBS 333.55)
6. *Lalaria communis* R.T. Moore (1990)
   Teleomorph: *Taphrina communis* (Sadebeck) Giesenhag
   Host: *Prunus americana* Marsh
   Origin: Mix (1954), nos. 27, 28, 29 (CBS 352.35)
7. *Lalaria confusa* R.T. Moore (1990)
   Teleomorph: *Taphrina confusa* (Atkinson) Giesenhag
   Host: *Prunus virginiana* L.; *P. v.* var. *demissa* Torrey
   Origin: Mix (1954), no. 39 (CBS 375.39)
8. *Lalaria dearnessii* R.T. Moore (1990)
   Teleomorph: *Taphrina dearnessii* Jenkins
   Host: *Acer rubrum* L.
   Origin: Mix (1954), no. 40 (CBS 334.55)
9. *Lalaria deformans* R.T. Moore (1990)
   Teleomorph: *Taphrina deformans* (Berkeley) Tulasne
   Host: *Prunus communis* L.; *P. persica* L.
   Origin: Mix (1954), nos. 41, 42 (CBS 356.35, MUCL 30957)
10. *Lalaria farlowii* R.T. Moore (1990)
    Teleomorph: *Taphrina farlowii* Sadebeck
    Host: *Prunus serotina* Ehrhart
    Origin: Mix (1954), nos. 45, 46 (CBS 376.39)
11. *Lalaria flavorubra* R.T. Moore (1990)
    Teleomorph: *Taphrina flavorubra* Ray
    Host: *Prunus besseyi* Bailey
    Origin: Mix (1954), nos. 47, 48 (CBS 377.39)
12. *Lalaria johansonii* R.T. Moore (1990)
    Teleomorph: *Taphrina johansonii* Sadebeck
    Host: *Populus tremuloides* Michaux
    Origin: Mix (1954), nos. 49, 50 (CBS 378.39)
13. *Lalaria letifera* R.T. Moore (1990)
    Teleomorph: *Taphrina letifera* (Peck) Saccardo
    Host: *Acer spicatum* Lamarck
    Origin: Mix (1954), no. 51 (CBS 335.55)
14. *Lalaria nana* R.T. Moore (1990)
    Teleomorph: *Taphrina nana* Johanson
    Host: *Betula nana* L.
    Origin: Mix (1954), no. 52 (CBS 336.55)
15. *Lalaria polystichi* R.T. Moore (1990)
    Teleomorph: *Taphrina polystichi* Mix
    Host: *Polystichum acrostichoides* (Michaux) Schott
    Origin: Mix (1954), nos. 53, S4 (CBS 379.39)
16. *Lalaria populi-salicis* R.T. Moore (1990)
    Teleomorph: *Taphrina populi-salicis* Mix
    Host: *Populus trichocarpa* Torrey & Gray
    Origin: Mix (1954), no. 58 (CBS 419.54)
17. *Lalaria populina* R.T. Moore (1990)
    Teleomorph: *Taphrina populina* Fries
    Host: *Populus nigra* L.
    Origin: Mix (1954), nos. 55, 56 (CBS 337.55, MUCL 30958)
18. *Lalaria pruni-subcordatae* R.T. Moore (1990)
    Teleomorph: *Taphrina pruni-subcordatae* (Zeller) Mix
    Host: *Prunus subcordata* Bentham
    Origin: Mix (1954), no. 62 (CBS 381.39)

19. *Lalaria purpurascens* R.T. Moore (1990)
    Teleomorph: *Taphrina purpurascens* Robinson
    Host: *Rhus copallina* L.
    Origin: Mix (1954), no. 63 (CBS 338.55)
20. *Lalaria robinsoniana* R.T. Moore (1990)
    Teleomorph: *Taphrina robinsoniana* Giesenhag
    Host: *Alnus rugosa* (Du Roi) Sprengel; *A. serrulata* (Aiton) Willdenow
    Origin: Mix (1954), nos. 64, 67, 69 (CBS 382.39, CBS 383.39)
21. *Lalaria tosquinetii* R.T. Moore (1990)
    Teleomorph: *Taphrina tosquinetii* (Westendorp) Tulasne
    Host: *Alnus glutinosa* Gaertner
    Origin: Mix (1954), nos. 74, 75 (CBS 276.28)
22. *Lalaria ulmi* R.T. Moore (1990)
    Teleomorph: *Taphrina ulmi* (Fuckel) Johanson
    Host: *Ulmus rubra* Muhlenberg
    Origin: Mix (1954), nos. 77, 78 (CBS 420.54)
23. *Lalaria virginica* R.T. Moore (1990)
    Teleomorph: *Taphrina virginica* Sadebeck
    Host: *Ostrya virginiana* (Miller) K. Koch
    Origin: Mix (1954), no. 79 (CBS 340.55)

Key to isolates

The following key is to all isolates studied by Mix (some of which have been recognized in *Lalaria*). This key points up the fact that several hosts have species complexes, particularly *Quercus* and *Betula*. The three isolates identified as *T. betulina* Rostrup (nos. 2–4) were originally separate species of *Taphrina*: respectively, *T. betulina* and *T. lapponica* Juel on *Betula intermedia* Thomas (one of which is CBS 417.54) and *T. turgida* on *B. pendula* Roth; the original classification is supported by the fact that they key out separately. The 16 isolates of *T. caerulescens* on *Quercus* (nos. 5–20) key out to 14 taxa. In the traditional practice of yeast taxonomy, each terminal entry might be considered a separate species. Only the two species for which cultures are available (*L. coccinea* and *L. caerulescens*) have been accepted in *Lalaria* (Moore 1990). This key has been constructed using Mix's (1954) data.

Key to the anamorphs of *Taphrina* and species of *Lalaria*: {Host} species and numbers are from Mix (1954). See Table 64.

1. a Galactose assimilated → 2
   b Galactose not assimilated → 28
2(1). a D-Ribose assimilated → 3
   b D-Ribose not assimilated → 14
3(2). a Galactitol assimilated → 4
   b Galactitol not assimilated → 8
4(3). a Soluble starch assimilated → 5
   b Soluble starch not assimilated → 6
5(4). a Melibiose assimilated .... *L. confusa*
   b Melibiose not assimilated .... *T. caerulescens* {*Quercus velutina*}: 18/19
6(4). a Ethanol assimilated .... *T. caerulescens* {*Q. maxima*}: 13/14
   b Ethanol not assimilated → 7
7(6). a Citric acid assimilated .... *T. caerulescens* {*Q. palustris*}: 16
   b Citric acid not assimilated .... *T. caerulescens* {*Q. bushii*}: 6
8(3). a D-Mannitol assimilated → 9
   b D-Mannitol not assimilated → 12
9(8). a Raffinose assimilated → 10
   b Raffinose not assimilated → 11
10(9). a Salicin assimilated .... *L. pruni-subcordatae*
   b Salicin not assimilated .... *L. communis*
11(9). a Salicin assimilated .... *L. virginica*
   b Salicin not assimilated .... *T. cerasi* {*Prunus pennsylvanica*}: 25/26
12(8). a L-Sorbose assimilated → 13
   b L-Sorbose not assimilated .... *L. nana*
13(12). a Melibiose assimilated .... *T. communis* {*Prunus* sp.}: 36/37
   b Melibiose not assimilated .... *T. communis* {*Pr. nigra*}: 35
14(2). a L-Arabinose assimilated → 15
   b L-Arabinose not assimilated → 23

Table 64
Key characteristics of species assigned to *Lalaria*

Species	Assimilation[a]																			Urease[b]	CoQ[c]
	Gal	Sor	Cel	Mel	Raf	SS	Xyl	Ara	Rbo	Eth	Gly	Rbi	Gat	Mnt	Glt	Sal	Suc	Cit	NO_3		
Lalaria americana	+	–	–	–	+	–	+	+	–	–	–	w	w	+	+	–	+	+	–		
L. caerulescens	–	–	+	–	–	–	+	–	w	–	+	+	–	+	+	+	+	–	+		
L. carnea	+	–	+	–	+	–	+	+	–	–	+	+	–	w	+	+	+	+	+	–	
L. cerasi	–	+	+	–	+	–	+	–	–	–	–	+	–	+	+	+	+	+	+		10
L. coccinea	–	w	+	–	w	–	+	+	+	+	+	+	+	+	+	+	+	+	+		
L. communis	+	+	+	–	+	–	+	+	+	–	+	+	–	+	+	–	+	+	+		
L. confusa	+	–	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+		
L. dearnessii	+	–	+	+	+	–	+	+	–	–	+	+	–	–	+	+	+	+	+		
L. deformans	–	+	–	–	–	–	+	–	–	–	+	+	–	–	+	w	+	+	+	–	10
L. farlowii	–	–	+	w	+	–	+	–	–	–	+	+	–	–	+	+	+	+	+		
L. flavorubra	–	–	+	–	+	–	+	–	w	–	+	w	–	–	+	w	+	+	+		
L. johansonii	–	–	+	–	–	–	+	–	–	–	+	–	–	+	+	+	+	+	+		
L. letifera	–	–	+	–	+	–	+	–	–	–	+	+	–	+	w	w	+	+	+		
L. nana	+	–	–	–	+	+	+	+	w	–	–	w	–	–	w	–	–	+	+		
L. polystichi	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+		
L. populi-salicis	–	–	+	–	–	–	+	–	–	–	–	–	–	+	+	–	+	–	+		
L. populina	–	–	+	–	–	–	+	–	–	–	w	–	–	+	+	+	+	–	+	–	10
L. pruni-subcordatae	+	–	+	–	+	–	+	+	+	+	+	w	–	+	w	+	+	–	+		
L. purpurascens	–	+	–	+	+	–	+	–	–	–	+	–	–	+	+	+	+	+	–		
L. robinsoniana	–	–	+	+	+	+	+	+	+	–	+	–	–	+	+	+	+	+	+		
L. tosquinetii	–	–	–	–	–	–	+	+	–	–	–	–	–	+	+	+	+	–	+		
L. ulmi	+	w	+	–	–	+	+	+	–	+	+	+	w	+	w	+	+	+	–		
L. virginica	+	+	+	–	–	+	+	+	+	–	+	+	–	+	+	+	+	–	+		

[a] Data from Mix (1953) (for NO_3), and Mix (1954) (others).
Abbreviations: Gal, galactose; Sor, L-sorbose; Cel, cellobiose; Mel, melibiose; Raf, raffinose; SS, soluble starch; Xyl, D-xylose; Ara, L-arabinose; Rbo, D-ribose; Eth, ethanol; Gly, glycerol; Rbi, ribitol; Gat, galactitol; Mnt, D-mannitol; Glt, D-glucitol; Sal, salicin; Suc, succinic acid; Cit, citric acid; NO_3, nitrate.
[b] Data from Moore and Flinn (1991) (spp. nos. 3, 17).
[c] Data from Yamada et al. (1983) (sp. no. 4), Yamada et al. (1987b) (sp. no. 9), and Moore and Flinn (1991) (spp. nos. 3, 17).

15(14). a Soluble starch assimilated → 16
b Soluble starch not assimilated → 19
16(15). a Melibiose assimilated → 17
b Melibiose not assimilated → 18
17(16). a Raffinose assimilated .... *T. sacchari* {*Acer saccharum*}: 70/71
b Raffinose not assimilated .... *T. betulina* {*Betula intermedia*}: 3
18(16). a Nitrate assimilated .... *T. ulmi* {*Ulmus alata*}: 76
b Nitrate not assimilated .... *L. ulmi*
19(15). a Cellobiose assimilated → 20
b Cellobiose not assimilated → 22
20(19). a Melibiose assimilated .... *L. dearnessi*
b Melibiose not assimilated → 21
21(20). a Raffinose assimilated .... *L. carnea*
b Raffinose not assimilated .... *T. caerulescens* {*Q. marilandica*}: 12
22(19). a L-Sorbose assimilated .... *T. caerulescens* {*Q. prinoides*}: 17
b L-Sorbose not assimilated .... *L. americana*
23(14). a Soluble starch assimilated → 24
b Soluble starch not assimilated → 27
24(23). a Glycerol assimilated → 25
b Glycerol not assimilated → 26
25(24). a L-Sorbose assimilated .... *T. communis* {*Pr. angustifolia*}: 30/31/32
b L-Sorbose not assimilated .... *T. confusa* {*Pr. virginiana*}: 38
26(24). a Citric acid assimilated .... *T. betulina* {*B. pendula*}: 4
b Citric acid not assimilated .... *T. potentillae* {*Potentilla arguta*}: 59
27(23). a Citric acid assimilated .... *T. epiphylla* {*Alnus incana*}: 43/44
b Citric acid not assimilated .... *T. caerulescens* {*Q. nigra*}: 15
28(2). a Raffinose assimilated → 29
b Raffinose not assimilated → 39
29(28). a L-Sorbose assimilated → 30
b L-Sorbose not assimilated → 35
30(29). a D-Ribose assimilated → 31
b D-Ribose not assimilated → 34
31(30). a L-Arabinose assimilated → 32
b L-Arabinose not assimilated → 33
32(31). a Melibiose assimilated .... *T. communis* {*Pr. maritima*}: 34
b Melibiose not assimilated .... *L. coccinea*
33(31). a Citric acid assimilated .... *T. communis* {*Pr. lanata*}: 33
b Citric acid not assimilated .... *T. caerulescens* {*Q. laurifolia*}: 10
34(30). a Melibiose assimilated .... *L. purpurascens*
b Melibiose not assimilated .... *L. cerasi*
35(29). a Melibiose assimilated → 36
b Melibiose not assimilated → 37
36(35). a Soluble starch assimilated .... *L. robinsoniana*
b Soluble starch not assimilated .... *L. farlowii*
37(35). a D-Mannitol assimilated → 38
b D-Mannitol not assimilated .... *L. flavorubra*
38(37). a D-Ribose assimilated .... *T. potentillae* {*Po. canadensis*}: 60/61
b D-Ribose not assimilated .... *L. letifera*
39(28). a Cellibiose assimilated → 40
b Cellobiose not assimilated → 44
40(39). a Ribitol assimilated → 41
b Ribitol not assimilated → 48
41(40). a Citric acid assimilated → 42
b Citric acid not assimilated → 43
42(41). a Melibiose assimilated .... *T. caerulescens* {*Q. ilicifolia*}: 8
b Melibiose not assimilated .... *T. caerulescens* {*Q. virginiana*}: 20
43(41). a Ethanol assimilated .... *T. caerulescens* {*Q. kelloggii*}: 9
b Ethanol not assimilated .... *L. caerulescens*
44(39). a L-Sorbose assimilated .... *L. deformans*
b L-Sorbose not assimilated → 45
45(44). a Citric acid assimilated → 46
b Citric acid not assimilated → 47
46(45). a Melibiose assimilated .... *T. betulina* {*B. intermedia*}: 2
b Melibiose not assimilated .... *T. caerulescens* {*Q. macrocarpa*}: 11
47(45). a L-Arabinose assimilated .... *L. tosquinetii*
b L-Arabinose not assimilated .... *L. polystichi*

48(40). a Glycerol assimilated → 49
b Glycerol not assimilated → 50
49(48). a Citric acid assimilated .... *L. johansonii*
b Citric acid not assimilated .... *L. populina*
50(48). a Salicin assimilated .... *T. populi-salicis* {*Populus fremontii*}: 57
b Salicin not assimilated .... *L. populi-salicis*

Comments on the genus

67.1. The teleomorph phase

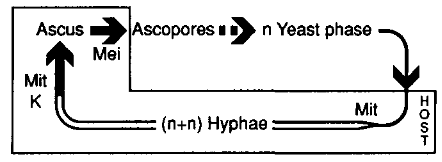
The order Taphrinales Gaumann comprises two families (see Kurtzman 1993a; p. 172):

- The Protomycetaceae (*Burenia* Reddy & Kramer, 2 spp.; *Protomyces* Unger, 10+ spp., *Protomycopsis* Magnus, 5 spp., *Taphridium* de Lagerheim & Juel, 2 spp., and *Volkartia* Maire, 1 sp.).
- The monotypic Taphrinaceae (*Taphrina* Fries, ~95 spp.).

Mixia osmundae (Nishida) Kramer, a fern parasite, was originally described in *Taphrina* before being given generic status in the Protomycetaceae (Kramer 1958) on the basis of the apparent similarity in spore formation and of the multinucleate condition of both the mycelium and the young fruiting structures; *T. higginsii* Mix, a similar parasite on a different species of *Osmunda,* was considered a synonym. *Mixia* was subsequently placed in its own family (Kramer 1987) because, unlike the rest of the Protomycetaceae, but like *Taphrina,* it formed a thin-walled sporocarp and the "asci" were subtended by empty stalk cells; Reddy and Kramer (1975) excluded it from the family and, in a dendrogram of possible phylogenetic relationships, placed it tentatively between *Volkartia* and the Mucorales. Nishida et al. (1995) have presented convincing evidence that the species is a basidiomycete: electron micrographs show ellipsoidal to fusiform sporogenous cells producing a number of stalk cells on their surface that generate spores blastically and not from within; consequently, the previously identified endospores (as ascospores and blastospores) are, in fact, formed externally. The 18S rRNA gene sequence also supports the basidiomycete interpretation.

The general life-cycle of the Protomycetaceae is shown in Fig. 278b. The mycelium is multinucleate, apparently diploid, and develops thick-walled, multinucleate ascogenous cells which either spread through the host tissue or form a continuous subepidermal layer (Reddy and Kramer 1975). Meiosis in the indurated cells, the asci, produces ascospores which initiate the saprobic, yeast haplophase; only diploid cells, resulting from cell and nuclear fusion, are capable of infection; host range is restricted to Umbelliferae and Compositae.

The only comprehensive monograph of *Taphrina* is that by Mix (1949). The approximately 95 species are classified entirely by teleomorphic characters: ascus morphology, location of the mycelium within the host tissue, histological effects on the host, and the host species. There are, however, many examples where morphological criteria overlap and host specificity is inconsistent. These fungi are all highly specialized plant parasites of limited host range and are found naturally only on their respective host plants where they cause malformations of the tissues attacked, producing such disease symptoms as leaf curl, puckering, pockets, blisters, and witches' broom. Ascospores are formed only in nature and bud readily, either within the ascus or after spore discharge. There is reason to believe (Mix 1935) that propagation by budding in the anamorphic phase may continue indefinitely, the cells surviving in this fashion on various plant surfaces and probably also in the soil.



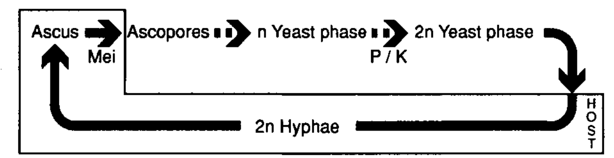


Fig. 278. Generalized life cycles in (a) the Taphrinaceae and (b) the Protomycetaceae. The mycelial parasitic phase is outlined; see the text for a full explanation. Abbreviations: K, karyogamy; Mei, meiosis, Mit, mitosis, P, plasmogamy.

The general life-cycle of the Taphrinaceae is shown in Fig. 278a. The parasitic, more or less coenocytic, dikaryotic mycelium grows and branches, penetrating the various cells and tissues of the host. Hyphal strands eventually break up into binucleate ascogenous cells that are often incorrectly called chlamydospores because of their somatic origin. It should also be noted that this dikaryon is more comparable to fruitbody-less ascogenous hyphae than to typical basidiomycete dikaryotic mycelia (Blanz and Unseld 1987). Karyogamy occurs within each cell and about this time the cells begin to elongate. The diploid nucleus divides mitotically while this elongation is proceeding, one daughter nucleus remaining near the base of the cell while the other moves towards the growing tip. A septum forms between the two diploid nuclei, dividing the cell into a stalk cell – whose protoplast disintegrates and leaves it empty – and an upper, ascus cell that forms eight primary ascospores (see Kramer 1960). The asci form a palisade layer above the epidermis and it is their presence which gives the surface its waxy bloom. The ascospores, or their buds, are projected from the unitunicate asci which often open by a characteristic slit. Ascospores occasionally undergo yeastlike budding while still in the ascus. Budding usually starts, however, after

spore discharge, producing numerous uninucleate, thin-walled, secondary spores. During summer and autumn, some secondary spores form yeastlike colonies on the twigs of the host. These colonies overwinter in crevices of the bark or between bud scales. The spores germinate on the young leaves, infecting them as the buds open. Most species are homothallic but *T. epiphylla* Sadebeck, at least, is strictly heterothallic; a few species, e.g., *T. deformans* and *T. pruni* Tulasne, may behave as heterothallics but are usually homothallic (see Gäumann 1952). The single haploid nucleus divides once and the paired daughter nuclei then pass into the germ tube which, on penetration of the host, gives rise to the more or less coenocytic, dikaryotic mycelium.

There are three types of mycelial habit (Mix 1949): (1) intercellular forms (e.g., *T. deformans*) develop abundant mycelium between the interior cells of leaf, stem, or fruit and subsequently form a subcuticular layer of ascogenous cells; (2) subcuticular forms (e.g., *T. betulina*) in which the mycelium and ascogenous cells grow only beneath the cuticle; (3) wall-inhabiting forms (e.g., *T. laurencia* Mix) that live entirely within the outer epidermal wall of the host.

The Taphrinales are usually interpreted as simple ascomycetes and have, as a rule, been classified in the Saccharomycetes (Hemiascomycetes), which mainly comprise the ascus-forming yeasts. More recently, the Taphrinales and similar species, termed archiascomycetes, have been found to form a lineage distinct from that of euascomycetes and hemiascomycetes (Nishida and Sugiyama 1994). Historically, some authors, however, have suggested that the Taphrinales are related to the basidiomycetes. Savile (1955, 1968) asserted that ancient parasitic fungi developed on primitive plants, e.g., ferns, and he therefore considered *Taphrina* to be close to primitive rusts, suggesting that they had a common ancestor, which he referred to as *Prototaphrina* (nom. nud.). Von Arx in 1967 (see von Arx et al. 1982) classified the Taphrinales, Exobasidiales, and Ustilaginales, all of which are dimorphic and have yeastlike states, with the Saccharomycetales (Endomycetales), later (von Arx 1979b, p. 567) he stated that he "... would prefer to classify the Taphrinales next to the Sporobolomycetales and the Ustilaginales in the Ustomycetes." Raper (1968) found Savile's (1968) hypothesis attractive, saying that various characteristics of the basidiomycetes could be rationalized from such an ancestor far more easily than from more highly differentiated ascomycetous forms. Syrop and Beckett (1972) tended to agree with Savile's (1955) interpretation that *Taphrina* was an "ancient organism" because the ascospore-delimiting membranes appeared to arise individually from the plasmalemma rather than by the invagination of an ascal sac; but, in as much as the spindle pole bodies were not preserved in potassium permanganate prepared material, it is not possible to critically compare *Taphrina* ascospore formation with, respectively, holocarpic and ascocarpic ascomycetes (see Moore 1987b). Prillinger et al. (1990c) state that the "Molecular and biochemical data (cell wall carbohydrate, 5S rRNA, ubiquinone (Q-9, Q-10), urease activity) position the Schizosaccharomycetaceae, the Protomycetaceae, and Taphrinaceae in the phylogenetic vicinity of the Basidiomycetes (e.g., genera of the *Microbotryum* [*Ustilago*]-type) and of the fruit-body forming Ascomycetes (e.g., *Ophiostoma* species)." Donk (1973b), while recognizing that some features of *Taphrina* were more common to the basidiomycetes, nevertheless rejected Savile's (1968) interpretation because the spores were produced endogenously.

The other genera in the order (see Reddy and Kramer 1975) are poorly understood and only a few of their species are in culture.

67.2. The anamorph phase

Lalaria, as presently circumscribed (Moore 1990), is the ordinal repository for the anamorphs of the Taphrinaceae Gaümann and the Protomycetaceae Gray. Although there are some 115 species in the order, only those 23 species included in the genus have available cultures, have been systematically studied, and conform to the generic description. There is a critical need for new cultures of all species, particularly those not presently in culture; these should be initiated from single ascospore isolates. I anticipate that future studies of these yeasts will find general characters suitable for establishing additional genera paralleling the teleomorphs. *Protomyces inundatus* Dangeard (Valadon et al. 1962), for example, has been found by Trinci and Gull (1973) to be sensitive to griseofulvin and by Ahmad (1973) to require thiamine for growth and to have the ability to assimilate ammonium acetate.

There have been a number of physiological and molecular biological studies of these yeasts, some of which present conflicting evidence as to whether the isolates are more like ascomycetes or basidiomycetes. Much of this equivocal data, undoubtedly, is in the nature of the fungi themselves but some of it, at least, is a consequence of accidentally isolating basidiomycetous yeasts from host substrates and erroneously labeling them as taphrinalean anamorphs. A brief synopsis of the available data follows, in alphabetical order, under the following headings: 1. Assimilation and morphology, 2. Auxins and cytokinins, 3. Budding, 4. Buffer-soluble proteins, 5. Cell walls, 6. Fatty acid ratios, 7. Mitosis morphology, 8. Pigmentation, 9. Polyols, 10. 5S rRNA nucleotide sequences, 11. Sterols, 12. Ubiquinones, and 13. Urease.

Assimilation and morphology: Verona and Rambelli (1962a,b) studied 21 isolates of *Taphrina* from the CBS Collection at Baarn, most of which were deposited by Mix. All of these isolates were non-fermentative and were able to assimilate glucose but not lactose. Most of the isolates were also able to assimilate xylose, maltose,

or mannitol. Among the isolates they identified one, which was labeled *T. californica* Mix, as *Cryptococcus laurentii.* Von Arx et al. (1982) reported that the *T. californica* isolate (CBS 374.39) had morphological characters that were slightly different from other *Taphrina* isolates; see the subsection on sterols. All of the other 19 isolates formed starch-like compounds, grew slowly, and would not grow at temperatures above 28°C; most of these isolates could assimilate nitrate. Verona and Rambelli (1962c) reported another *Taphrina* isolate to be a *Candida* sp. They proposed *Saprotaphrina* nom. nud. for the anamorphs of *Taphrina* (Verona and Rambelli 1962a). Unfortunately, they did not designate a type species and the isolates chosen were not identified by their CBS numbers.

Auxins and cytokinins: Kern and Naef-Roth (1975) examined ten species and found that all synthesized indole-3-acetic acid (IAA) and indole-lactic-acid (ILA) from tryptophan; traces of tryptophol were formed only by *T. betulae* and *T. betulina.* All culture filtrates lacked tryptamin and were high in cytokinin activity due to the presence of zeatin, IPA, and a third, unidentified substance.

Budding: Von Arx and Weijman (1979) comment, without documentation, that basipetal, enteroblastic budding was observed in *T. californica, T. deformans, T. polystichi* and *Protomyces macrosporus* as well as *Symbiotaphrina buchneri* and *S. kochii* (see section 67.3). Von Arx et al. (1982) interpreted budding in *Taphrina* to be rather similar to that of the apiculate yeasts and concluded that the genera *Taphrina, Mixia,* and *Protomyces* were correctly classified as hemiascomycetes. Electron micrographs of *T. californica* (CBS 374.39) bud scars by Heath et al. (1982) clearly show the flared multiple wall layers characteristic of basidiomycete budding.

Buffer-soluble proteins: Snider and Kramer (1974a,b) analysed 31 isolates of *Taphrina* by polyacrylamide gel electrophoresis (PAGE). They found that no one band occurred in all samples, although some fractions appeared to be characteristic of the genus in both mobility and concentration of the proteins. When isolates were grouped on the basis of host families, seven of the eight host families had percentages of mean homology. Only one family, the Polypodiaceae, was lower than the norm. When isolates were grouped according to the ascus morphologies, one out of six, which contained the species of family Polypodiaceae, was lower than the norm. These findings tend to support the concepts of conventional taxonomy, except possibly for the *Taphrina* species grouped in the host family Polypodiaceae.

Cell walls: Petit and Schneider (1983) analysed a strain of *T. deformans* that they isolated from spores of a single ascus on a peach leaf using a micromanipulator. Their results showed that there was a heteropolysaccharide fraction that, in four-day-old cultures, contained 54% mannose, 31% galactose, and 15% rhamnose; that chitin, if present, formed no more than 2% of the total wall composition; and that glucosamine was the only amino sugar. They stated that the biochemical profile they obtained was most like that of *Schizosaccharomyces.*

Fatty acid ratios: Watson and Kramer (1974) analysed 19 isolates of *Taphrina* grouped according to host family and host genera. They found no significant patterns indicative of phylogenetic relationships. *Taphrina* does, however, have relatively slow growth rates and the fatty acid ratios may change during maturation.

Mitosis morphology: The potential of mitotic analysis to help explain relationships among hypothetically primitive basidiomycetes was demonstrated by Heath and Heath (1976) who showed that the cowpea rust has a mitotic apparatus that shares characteristics with those of some ascomycetes. Heath et al. (1982) similarly studied isolates identified as from five species of *Taphrina.* They concluded that those of three species – *T. cerasi, T. faulliana* Mix, and *T. polystichi* – were typical ascomycetes. Each of two isolates of the fourth species, *T. deformans,* was apparently a mixed culture because, although ascomycete-type mitosis occurred in cells of both isolates, one isolate also had cells with another kind of ascomycete mitotic system characterized by elaborate and unique nucleus-associated organelles, whereas the second isolate had, in addition, cells that contained a basidiomycetous mitotic apparatus. The isolate from the fifth species, *T. californica,* contained a typical basidiomycetous mitotic system as well as basidiomycete-type bud scars; both of these traits indicate that the isolate (CBS 374.39) is misidentified. The detailed differences that they found among the mitotic systems of the true *Taphrina* isolates suggests that the genus as a whole may be polyphyletic but that it is undoubtedly ascomycetous. For three of the isolates, they found a previously unreported pattern of spindle formation otherwise known only in the red algae, a finding that could be used to support the hypothesis of a red algal ancestry for the ascomycetes (see Moore 1989a). Heath et al. (1987) extended the previous study showing that the isolate of *T. maculans* Butler had the basidiomycete pattern whereas three species of *Protomyces* (*P. inouyci* P. Hennings, *P. lactucae-debilis* Sawada, and *P. pachydermus* Thuem.) collectively had a mitotic apparatus that was clearly similar to the typical ascomycete type.

Pigmentation: Tubaki (1957) made ethanol extracts of isolates of *Taphrina* and *Protomyces* and examined them spectrophotometrically; his results showed that "... members of the two genera resemble each other closely as to pigmentation. Though colonies of these fungi are pinkish in color, none of them produced the carotenoid pigments which are characteristic of *Rhodotorula.*" Valadon (1964) using improved techniques found, however, that isolates of *Protomyces* did contain carotenoids whereas those of *Taphrina* did not, and he

suggested that pigment characteristics of certain fungi may be an aid in elucidating their taxonomic affinities. Van Eijk and Roeymans (1982) screened isolates of *Taphrina* and *Protomyces* for the presence of carotenoid pigments and the sterols ergosterol and brassicasterol. They concluded that the two genera cannot be separated on the basis of the presence or absence of carotenoids. All the *Taphrina* isolates were found to produce carotenoids though in a lower concentration than those of *Protomyces*.

Polyols: Rast's group has extensively investigated these acyclic sugar alcohols. On the basis of their studies, which included species of *Taphrina*, they recommended that the Endomycetales be assigned a rank adequate to its distinction (Rast and Pfyffer 1989). They also reported (Pfyffer et al. 1990) that *Taphrina*, in accumulating mannitol, behaves perfectly as a euascomycete (all of which are the P_2-type) and stated: "The P_2-type of *Taphrina* thus supports the hypotheses that the Taphrinales are among the oldest true Ascomycetes, ... and, in possessing the largest combination of common characteristics, befit also a likely ancestor of the Basidiomycetes ... ".

5S rRNA nucleotide sequences: Walker and Doolittle (1982) showed that sequences in the smut fungi have uridine in the 107 position but that this position is vacant in other Basidiomycota. Walker and Doolittle (1983) subsequently identified five clusters. Clusters 1 and 2 had a type A molecule with a "U" in the 107 position while clusters 3–5 had the type B molecule with an empty 107 position. Gottschalk and Blanz (1985) produced a dendrogram showing the relationships between the basidiomycetous fungi based on the 5S rRNA sequences. They stated that the 5S rRNA from *T. deformans* corresponded best with those sequences found in the basidiomycetes and placed it on its own next to Cluster 1 which contains a number of smut-like species (see Moore 1988a); but it was also starred to indicate that it corresponded "with the type A/type B bifurcation only in part." In an addendum at the end of the paper they cited a recent paper by Walker (1985a) which contained an analysis of *Protomyces inundatus* that showed it had 5S rRNA very close to that of *T. deformans*. In a subsequent paper (Blanz and Gottschalk 1986) the diagram appeared without *T. deformans*, but it is reinstated by Blanz and Unseld (1987). They also reported a dichotomy that has been found in 28S rRNA: an insertion of 27 nucleotides that occurs between positions 603 and 604 in *Exobasidium vaccinii* (Fuckel) Woronin, *Rhodosporidium toruloides*, *Septobasidium carestianum* Bresadola, and *Ustilago hordei* (Persoon:Persoon) de Lagerheim, but that is absent from *Saccharomyces carlsbergensis*, *Schizosaccharomyces pombe*, *T. deformans*, and *T. vestergrenii* Gies.

Sterols: Van Eijk and Roeymans (1982) screened isolates of *Taphrina* and *Protomyces* for the presence of ergosterol and brassicasterol. They found that 30 strains (24 spp.) of *Taphrina* produced brassicasterol as the principal sterol, whereas in four isolates (4 spp.), no brassicasterol could be detected (*T. californica*, *T. cerasi*, *T. farlowii* and *T. maculans*). In these latter isolates ergosterol was found to be the major component, while it was missing or only slightly present in the remaining *Taphrina* species. These authors concluded that there was no reason to doubt the identification of the four *Taphrina* isolates which did not form brassicasterol (but see the subsections on budding and on mitosis morphology). The effects of sterol inhibitors on *T. deformans* have been reported by Weete et al. (1985) and Sancholle et al. (1988).

Ubiquinones: The ubiquinone Q-10 system, otherwise generally restricted to basidiomycete yeasts, has been reported from isolates of *Protomyces inouyei*, *P. lactucae-debilis*, *P. pachydermus*, *T. caerulescens*, *T. californica*, *T. carnea*, *T. deformans*, *T. maculans*, *T. populina*, *T. tormentillae* Rostrop, and *T. wiesneri* (Rathay) Mix (Yamada et al. 1983, 1987b, Sugiyama et al. 1985, Moore and Flinn 1991).

Urease: Moore and Flinn (1991) reported positive results for *T. californica*, *T. maculans*, *T. tormentillae*, *Symbiotaphrina buchneri*, and *S. kochii* (weak) and negative results for *Lalaria carnea*, *L. deformans*, and *L. populina*.

67.3. *Saitoella*, *Neolecta* and *Symbiotaphrina*

The genus *Saitoella* (Goto et al. 1987a, Sugiyama et al. 1993) is represented by the single species *S. complicata*, an anamorph species that was described to accommodate two isolates of Himalayan soil yeasts that had been identified as *Rhodotorula glutinis*. Its basidiomycetous characters include enteroblastic budding (confirmed by electron micrographs), Co-Q 10, colonies that are yellow orange because of carotinoid pigments, and a positive urease reaction. The authors classified it in the Cryptococcaceae but considered it to be ascomycetous because they found it to be DBB negative and to lack xylose in the cell walls. They also said that they believed the genus had a probable close relationship with the Taphrinales because of the colony pigmentation and the cell wall biochemistry. Nishida and Sugiyama (1994), using 18S rRNA, found *Saitoella* to group with *Protomyces*, *Schizosaccharomyces* and *Taphrina*. *Saitoella* differs from *Lalaria* in not forming starch-like compounds, being urease positive, and in having enteroblastic budding.

The genus *Symbiotaphrina* Kühlwein & Jurzitza ex Gams & von Arx (1980) has been attributed to the Taphrinales because it only "... differs from *Taphrina* [anamorphs] by its symbiotic mode of life." There are two species, both of which occur in intestinal mycetomata of Anobiid beetles (Jurzitza 1979): the type, *S. buchneri* Grabner ex Gams & von Arx from *Stegobium paniceum* (L.) Jacobson (= *Sitodrepa panicea* (L.) Leconte) and *S. kochii* Jurzitza ex Gams & von Arx from *Lasioderma serricorne* F. The extensive studies of endosymbiosis (Buchner 1965) show the insect hosts to be

dependent on the yeasts for essential growth factors and to have evolved very sophisticated behavior patterns to assure the generational transfer of the yeasts. Both species of *Symbiotaphrina* are exceptional in their inability to assimilate urea (Kühlwein and Jurzitza 1961, Jurzitza 1964, Bismanis 1976) and they further differ generically from the species of *Lalaria* in being able to assimilate erythritol and L-rhamnose (but see Bismanis 1976), and in lacking any known teleomorph. Noda and Kodama (1996), using small-subunit rRNA, found the two species to be distinct from each other and to form a distinct line close to the discomycetes. There are also yeastlike symbiotes in the fat bodies of three genera of rice planthoppers; Noda et al. (1995), using 18S rRNA, found these fungi to be monophyletic and related to the pyrenomycetes.

The teleomorph genus *Neolecta* (Landvik 1996), on the basis of rDNA analyses, is believed to be close to the basal ascomycetes; it clusters with the Schizosaccharomycetales, Taphrinales (Taphrinaceae and Protomycetaceae), and Pneumocystidales and with these is thought to constitute a sister group to the Saccharomycetales and to all other ascomatous ascomycetes.

68. *Myxozyma* van der Walt, Weijman & von Arx

C.P. Kurtzman

Diagnosis of the genus

Vegetative reproduction is by multilateral budding on a narrow base. Cells are spheroidal or ellipsoidal. Rudimentary pseudohyphae may be present but true hyphae are not formed.

Sugars are not fermented. Nitrate is not assimilated. Cultures are often quite mucoid. Extracellular starch-like compounds are produced. Diazonium blue B reaction is negative.

Type species

Myxozyma melibiosi (Shifrine & Phaff) van der Walt, Weijman & von Arx

Species accepted

1. *Myxozyma geophila* van der Walt, Y. Yamada & Nakase (1987)
2. *Myxozyma kluyveri* van der Walt, Spencer-Martins & Y. Yamada (1989)
3. *Myxozyma lipomycoides* van der Walt, Y. Yamada & Nakase (1987)
4. *Myxozyma melibiosi* (Shifrine & Phaff) van der Walt, Weijman & von Arx (1981)
5. *Myxozyma monticola* Pretorius & Spaaij (1993)
6. *Myxozyma mucilagina* (Phaff, Starmer, Miranda & M.W. Miller) van der Walt, Weijman & von Arx (1981)
7. *Myxozyma udenii* Spaaij, Weber, Oberwinkler & van der Walt (1990)
8. *Myxozyma vanderwaltii* Spaaij, Weber & M.Th. Smith (1993)

Key to species

See Table 65.

1.	a	Melezitose assimilated → 2		
	b	Melezitose not assimilated → 5		
2(1).	a	L-Rhamnose assimilated → 3		
	b	L-Rhamnose not assimilated → 4		
3(2).	a	Melibiose assimilated	*M. monticola*:	p. 595
	b	Melibiose not assimilated	*M. mucilagina*:	p. 595
4(2).	a	D-Arabinose assimilated	*M. vanderwaltii*:	p. 596
	b	D-Arabinose not assimilated	*M. udenii*:	p. 596
5(1).	a	Cellobiose assimilated → 6		
	b	Cellobiose not assimilated	*M. geophila*:	p. 593
6(5).	a	Trehalose assimilated → 7		
	b	Trehalose not assimilated	*M. melibiosi*:	p. 594
7(6).	a	Melibiose assimilated	*M. kluyveri*:	p. 593
	b	Melibiose not assimilated	*M. lipomycoides*:	p. 594

Table 65
Key characters of species in the genus *Myxozyma*

Species	Assimilation					
	Melezitose	L-Rhamnose	Melibiose	D-Arabinose	Cellobiose	Trehalose
Myxozyma geophila	–	–	–	+	–	–
M. kluyveri	–	+	+	+	+	+
M. lipomycoides	–	+	–	+	+	+
M. melibiosi	–	–	+	+	+	–
M. monticola	+	+	+	+	–	+
M. mucilagina	+	+	–	v	+	v
M. udenii	+	–	–	–	+	+
M. vanderwaltii	+	–	–	+	+	+

Systematic discussion of the species

68.1. *Myxozyma geophila* van der Walt, Y. Yamada & Nakase (van der Walt et al. 1987c)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.2–5.1)×(2.4–5.6) µm, and single or in pairs. Growth is mucoid, glistening and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, growth under the coverglass is devoid of pseudohyphae and true hyphae. A few outgrowths of undifferentiated cells are occasionally seen. Aerobic growth is mucoid, glistening, with entire margins, and tannish-white in color. A faint acidic, musky odor is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+/w	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	+
Lactose	v	D-Mannitol	s
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	s
D-Xylose	+	Succinate	s
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Gelatin liquefaction	+
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8 (van der Walt et al. 1987c).

Mol% G+C: 46.0, 46.8, CBS 7219, CBS 7037, respectively (T_m: van der Walt et al. 1987c).

Origin of the strains studied: CBS 7219 (NRRL Y-17252), CBS 7037 (NRRL Y-17246), soil, Transvaal, South Africa.

Type strain: CBS 7219.

Comments: Results from the present study are in agreement with the original description of this species (van der Walt et al. 1987c), except that sucrose assimilation was found to be weak or positive rather than negative.

68.2. *Myxozyma kluyveri* van der Walt, Spencer-Martins & Y. Yamada (van der Walt et al. 1989e)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.0–4.1)×(2.1–5.1) µm, and single or in pairs (Fig. 279). Growth is mucoid, glistening and tannish-white in color.

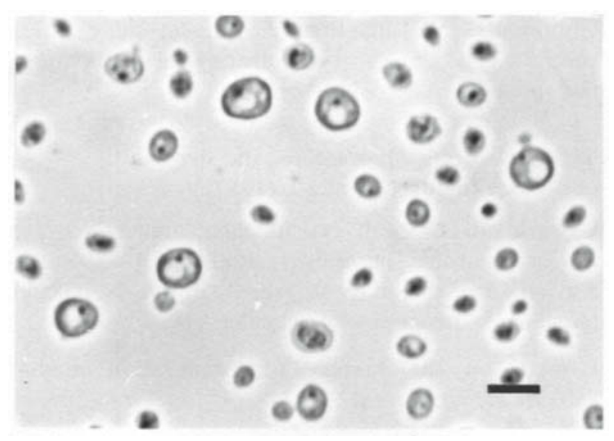


Fig. 279. *M. kluyveri*, CBS 7332. Budding cells after 3 days on 5% malt extract agar at 25°C. Bar = 5 µm.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither pseudohyphae nor true hyphae are formed under the coverglass. Aerobic growth is mucoid, glistening, with an entire margin, and tannish-white in color. There is a faint acidic, musky odor.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	s
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+
Inulin	v	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	s	Inositol	v
D-Ribose	s	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+/w
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8 (van der Walt et al. 1987c).

Mol% G+C: 39.5, CBS 7332 (T_m: van der Walt et al. 1989e).

Origin of the strains studied: CBS 7332 (NRRL Y-17277), CBS 7405 (NRRL Y-17359), soil, Transvaal, South Africa.

Type strain: CBS 7332.

Comments: Results from the present study are in generally good agreement with the original description of this species (van der Walt et al. 1989e).

68.3. *Myxozyma lipomycoides* van der Walt, Y. Yamada & Nakase (1987c)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.1–6.0)×(2.5–6.0) µm, and single or in pairs. Growth is mucoid, glistening and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither pseudohyphae nor true hyphae are formed under the coverglass. Aerobic growth is mucoid, glistening, and with entire to scalloped margins. The growth is tannish-white in color. A faintly acid, "metallic" odor is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	s	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	–
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	+	Citrate	s
D-Arabinose	s	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	w
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 9, CBS 7038 (van der Walt et al. 1987c).
Mol% G+C: 40.9, CBS 7038 (T_m: van der Walt et al. 1987c).

Origin of the strain studied: CBS 7038 (NRRL Y-17253), from a tree-inhabiting lichen, Transvaal, South Africa.

Type strain: CBS 7038.

Comments: Several differences in assimilation of carbon compounds were found between the present study and the original description (van der Walt et al. 1987c). In the present study, D-mannitol was not assimilated, whereas growth occurred with D-ribose, cellobiose and citrate.

68.4. *Myxozyma melibiosi* (Shifrine & Phaff) van der Walt, Weijman & von Arx (1981)

Synonyms:
Torulopsis melibiosum Shifrine & Phaff (1956)
Paratorulopsis melibiosi (Shifrine & Phaff) Novák & Zsolt (1961)
Cryptococcus melibiosum (Shifrine & Phaff) Phaff & Fell (1970)
Candida melibiosophila Golubev (1981)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (2.2–6.9)×(2.7–6.9) µm, and single, in pairs, or occasionally in small clusters (Fig. 280). Growth is mucoid, glistening and tannish-white in color.

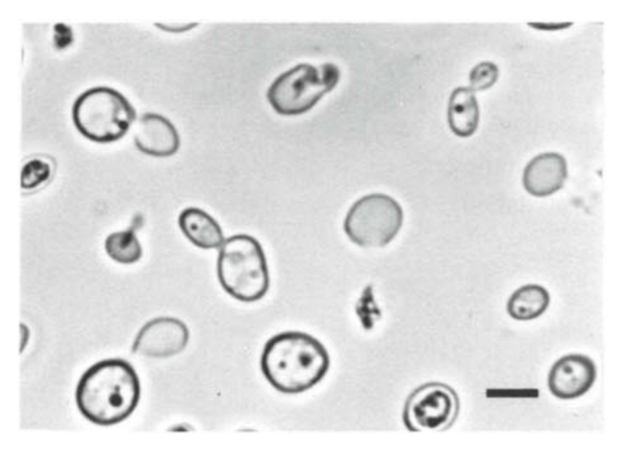


Fig. 280. *M. melibiosi*, CBS 2102. Budding cells, after 3 days on 5% malt extract agar at 25°C. Bar = 5 µm.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither pseudohyphae nor true hyphae are formed under the coverglass. Aerobic growth is mucoid, glistening, with entire to scalloped margins, and a tannish-white color. A faintly acidic odor with a trace of esters is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	v	D-Mannitol	w/–
Melibiose	+	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	s
Inulin	–	D-Gluconate	+/w
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	w
5-Keto-D-gluconate	s	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8, CBS 2102 (Yamada 1986a).
Mol% G+C: 50.3, CBS 2102 (T_m: van der Walt et al. 1987c).

Origin of the strains studied: CBS 2102 (NRRL Y-11781), CBS 2667 (NRRL Y-17360), both from the beetle *Dendroctonus monticolae* in ponderosa pine (*Pinus ponderosa*), California, U.S.A.

Type strain: CBS 2102.

68.5. *Myxozyma monticola* Pretorius & Spaaij (Pretorius et al. 1993)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.0–5.0)×(2.5–5.0) µm, and single or in pairs. Growth is mucoid, glistening and light tan in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither pseudohyphae nor true hyphae are formed under the coverglass. Aerobic growth is glistening, mucoid, with entire colony margins, and tannish-white in color. A faintly acidic odor is present.

Fermentation: absent.

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	s
Melibiose	+	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	s
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8 (Pretorius et al. 1993).
Mol% G+C: 45.2 (T_m: Pretorius et al. 1993).

Origin of the strains studied: CBS 7790 (NRRL Y-17725), from flux of an oak (*Quercus* sp.), South Africa; CBS 7806 (NRRL Y-17726), soil, South Africa.

Type strain: CBS 7806.

Comments: Results from the present study are in generally good agreement with the original description (Pretorius et al. 1993). Exceptions are growth on cellobiose, negative in the present study, and 5-keto-D-gluconate, positive in the present study.

68.6. *Myxozyma mucilagina* (Phaff, Starmer, Miranda & M.W. Miller) van der Walt, Weijman & von Arx (1981)

Synonym:
Candida mucilagina Phaff, Starmer, Miranda & M.W. Miller (1980)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ellipsoidal, (1.9–6.8)×(2.1–6.8) µm, and single, in pairs, or occasionally in clusters (Fig. 281). Growth is mucoid, glistening and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither hyphae nor pseudohyphae are formed under the coverglass. Aerobic growth is mucoid, glistening, with entire colony margins, and tannish-white in color. A faintly acidic odor is present.

Fermentation: absent.

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w/–
D-Xylose	+	Succinate	+/w
L-Arabinose	+	Citrate	+/w
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 8 (Phaff et al. 1980).
Mol% G+C: 43.2–44.0, CBS 7071 and 4 additional strains (BD: Phaff et al. 1980).

Origin of the strains studied: CBS 7071 (NRRL Y-11823, UCD-FST 76-236C), CBS 7070 (NRRL Y-17361, UCD-FST 76-234C), both from rotting agria cactus (*Stenocereus gummosis*), Baja California Sur, Mexico.

Type strain: CBS 7071.

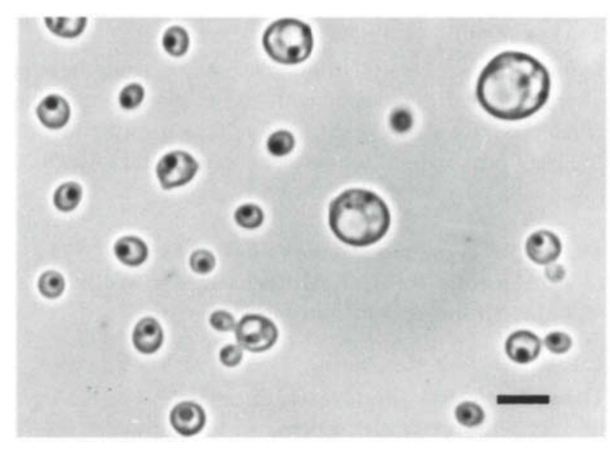


Fig. 281. *M. mucilagina*, CBS 7071. Budding cells after 3 days on 5% malt extract agar at 25°C. Bar = 5 µm.

68.7. *Myxozyma udenii* Spaaij, Weber, Oberwinkler & van der Walt (1990)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (3.0–5.6) × (3.0–6.2) µm, and single or in pairs. Growth is mucoid, glistening and tannish-white in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither hyphae nor pseudohyphae are present under the coverglass. Aerobic growth is mucoid, glistening, with smooth colony margins, and tannish-white in color. A faintly acidic odor is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	s	Galactitol	–
Lactose	+	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	w/s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w/s
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	+
10% NaCl/5% glucose	–		

Co-Q: 8 (Spaaij et al. 1990).
Mol% G + C: 49.2, CBS 7439 (T_m: Spaaij et al. 1990).

Origin of the strain studied: CBS 7439 (NRRL Y-17387), from soil around the roots of a mango tree (*Mangifera indica*), Miami, Florida, U.S.A.

Type strain: CBS 7439.

Comments: Results from the present study are in agreement with those of the original description (Spaaij et al. 1990).

68.8. *Myxozyma vanderwaltii* Spaaij, Weber & M.Th. Smith (1993b)

Growth on 5% malt extract agar: After 3 days at 25°C, the cells are spheroidal to ovoidal, (3.5–6.5) × (4.0–6.5) µm, and single or in pairs. Growth is mucoid, glistening and light tan in color.

Growth on the surface of assimilation media: Pellicles are not formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, neither hyphae nor pseudohyphae are evident under the coverglass. Aerobic growth is mucoid, glistening, with colony margins entire, and tannish-white in color. A faintly acidic, buttery odor is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	s	Galactitol	–
Lactose	v	D-Mannitol	–
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	s
D-Xylose	+	Succinate	v
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 8 (Spaaij et al. 1993b).
Mol% G + C: 44.8 (T_m: Spaaij et al. 1993b).

Origin of the strains studied: CBS 7793 (NRRL Y-17727), from a flower of *Protea repens* (L.)L, South Africa; CBS 7794 (NRRL Y-17728), from the flux of an oak (*Quercus* sp.), South Africa.

Representative strain: CBS 7793, a living ex-type strain is not available.

Comments: Results from the present study are in generally good agreement with the original description

(Spaaij et al. 1993b). Growth reactions on 2-keto-D-gluconate and 5-keto-D-gluconate were exceptions and found to be positive in the present study.

Comments on the genus

The genus *Myxozyma* was described by van der Walt et al. (1981) for anamorphic ascomycetous yeasts with physiological and cultural properties similar to *Lipomyces,* i.e., formation of extracellular starch-like compounds and growth on imidazol and imidazol-4-carboxylic acid as sole sources of nitrogen. The proposed close relatedness of *Myxozyma* and *Lipomyces* was confirmed by Kurtzman and Liu (1990) from analysis of partial sequences of small and large subunit rRNAs. However, with the exception of *M. kluyveri,* which clustered with *Lipomyces* species, the two genera appear as closely related but distinct groups. The rRNA comparisons indicated that the five species (*M. geophila, M. kluyveri, M. lipomycoides, M. melibiosi* and *M. mucilagina*) included in the study were genetically isolated from one another as proposed by Yamazaki and Goto (1985) and Yamada and Aizawa (1987) from analysis of isoenzyme mobilities. In addition to the preceding, various physiological and electrokaryotypic studies of the Lipomycetaceae have been reported by Botha and Kock (1993), Cottrell and Kock (1989, 1990), Kock et al. (1992), van der Walt (1992) and van der Walt et al. (1990a).

Validly described species not examined because of unavailability of living cultures: The following three species of *Myxozyma* were validly described by virtue of deposit of dried, dead holotypes in herbaria, but the authors, in contradiction to the intent of the Botanical Code (Greuter et al. 1994), did not distribute the living cultures characterized in their publications, thus preventing verification of physiological and genetic properties:

- *Myxozyma neotropica* Spaaij & Weber (Spaaij et al. 1992b);
- *Myxozyma nipponensis* Spaaij & Weber (Spaaij et al. 1993a);
- *Myxozyma sirexii* Spaaij & Weber (Spaaij et al. 1992a).

69. *Oosporidium* Stautz

M.Th. Smith

Diagnosis of the genus

Vegetative reproduction is by multilateral budding on a broad base combined with septation. Cells vary in shape and usually remain attached to each other, forming long chains. True septa are sometimes present and independent from the broad base budding. Arthrospores are absent. Asexual endospores are formed by protoplasmic cleavage.

Sexual reproduction absent.

Fermentation is absent. Nitrate is assimilated. Pink or orange-yellow pigments of a non-carotenoid nature are produced. Diazonium blue B reaction is negative.

Type species

Oosporidium margaritiferum Stautz

Species accepted

1. *Oosporidium margaritiferum* Stautz (1931)

Systematic discussion of the species

69.1. *Oosporidium margaritiferum* Stautz (1931)

Synonym:
Trichosporon margaritiferum (Stautz) Buchwald (1939)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, there is no visible growth. After one week, a granular or flocculent sediment is formed. Cells are globose and ovoidal or elongate, (5.0–12.0)×(6.0–12.5) µm. Vegetative reproduction is multilateral, usually by budding on a broad base combined with septation. Cells usually remain attached to each other and form long branched chains (Fig. 282). After one month a sediment is present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is light pink to light orange, dull, rugose, raised, and with a fragile surface.

Dalmau plate culture on corn meal agar: Pseudomycelium is present. It consists of branched chains of ovoid, globose or elongate cells. At the periphery of the colony, cells tend to become progressively more elongated (Fig. 283). True septa are sometimes formed.

Formation of asexual spores: Asexual endospores are formed by protoplasmic cleavage (Fig. 284). Cells are of variable size and shape and contain one to ten endospores

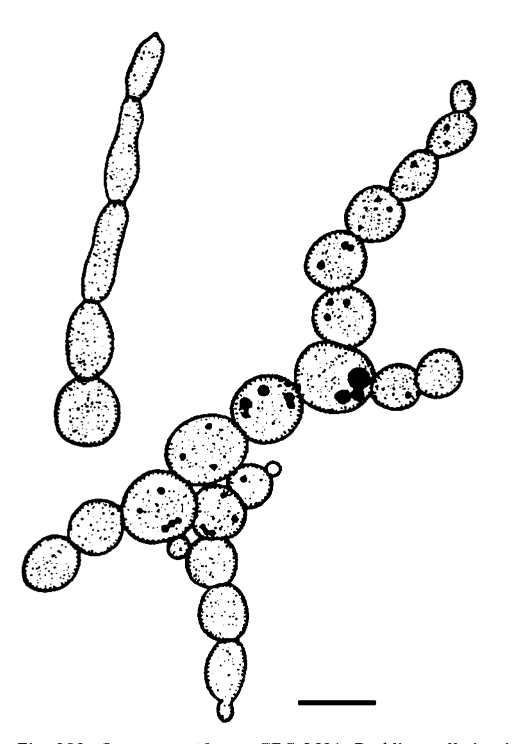


Fig. 282. *O. margaritiferum*, CBS 2531. Budding cells in glucose–yeast extract–peptone water after 1 week at 25°C. Bar = 10 µm.

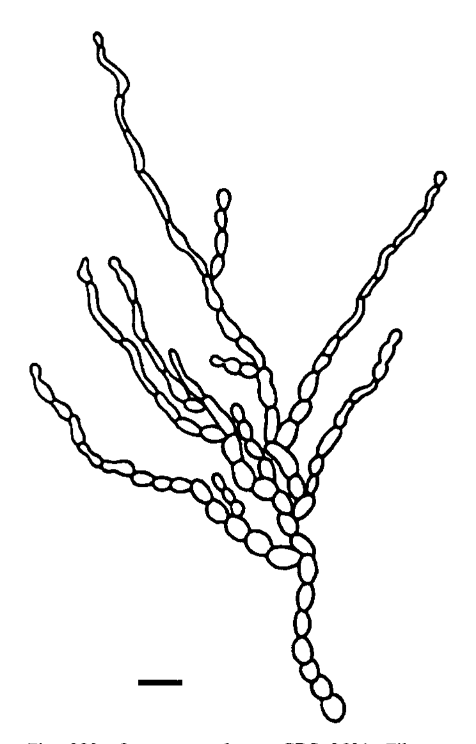


Fig. 283. *O. margaritiferum*, CBS 2531. Filaments in Dalmau plate culture on corn meal agar after 10 days at 25°C. Bar = 10 µm.

of various sizes that are globose or ellipsoidal in shape. Germination of endospores often starts inside the parent cell. Endospores were observed on YM and Gorodkowa agar slants and slide cultures after 4–6 weeks at 18–20°C and in slide cultures on corn meal and potato–dextrose agars. The slants had been inoculated with cultures grown in 15% liquid malt extract for 5–6 days at 25°C on a shaker (do Carmo-Sousa 1970b).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	n
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	n
Soluble starch	+	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	v	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

50% Glucose –
Maximum growth temperature 32–34°C.
Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 2531, slime flux, Stautz; CBS 6177, slime flux of elm tree, California, U.S.A., do Carmo-Sousa.

Type strain: CBS 2531, isolated by Stautz.

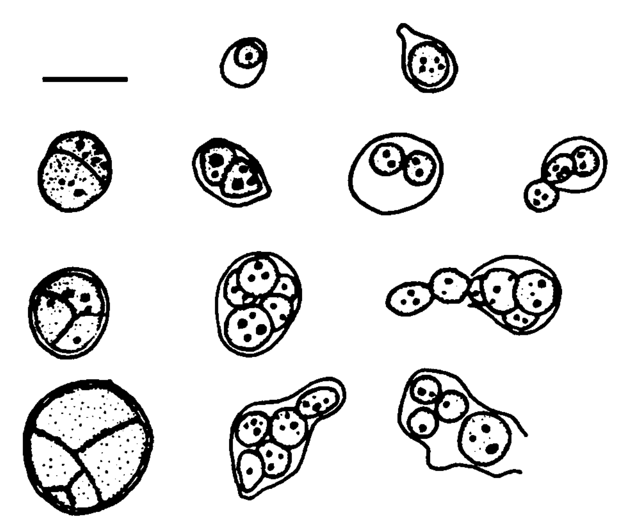


Fig. 284. *O. margaritiferum*, CBS 2531. Endospores on YM agar after 5 weeks at 25°C. Bar = 10 μm.

Comments: Strains of this species do not grow in the liquid media used for growth tests. Assimilation of carbon compounds was tested on agar slants of Yeast Nitrogen Base. Assimilation of nitrate was tested on agar slants of Yeast Carbon Base. Even on these solid media, growth was very slow.

The occurrence of broad-based budding and formation of pink to orange pigments are unusual characters for ascomycetous yeasts. A phylogenetic analysis of rDNA sequences placed *O. margaritiferum* among the euascomycetes (Kurtzman and Robnett 1995).

70. *Saitoella* S. Goto, Sugiyama, Hamamoto & Komagata

D.G. Ahearn, J. Sugiyama and R.B. Simmons

Diagnosis of the genus

Asexual reproduction is mainly by multipolar enteroblastic budding. Vegetative cells are ovoidal to ellipsoidal and occur singly or in pairs. True mycelium and pseudomycelium are normally absent. Ascospores, teliospores, and ballistospores are not formed.

Sugars are not fermented. Nitrate is assimilated. Starch-like compounds are not synthesized. Carotenoid pigments are formed. Xylose is absent in the cell walls. Diazonium blue B reaction is negative. The major ubiquinone system is Q-10.

Type species

Saitoella complicata S. Goto, Sugiyama, Hamamoto & Komagata

Species accepted

1. *Saitoella complicata* S. Goto, Sugiyama, Hamamoto & Komagata (1987)

Systematic discussion of the species

70.1. *Saitoella complicata* S. Goto, Sugiyama, Hamamoto & Komagata (1987a)

Growth in morphology broth: After 3 days at 20°C, the cells are mainly ovoidal to ellipsoidal, (2.5–8.0)×(3.0–14.0) μm; elongate pseudohyphal-type cells are occasionally present (Fig. 285A). Budding is mainly enteroblastic; the primary bud is holoblastic and the secondary, and probably successive buds, are enteroblastic. The cell wall is of the ascomycetous type (Fig. 285B,C). There is an orange-red ring and sediment. Pellicles are not formed.

Growth on morphology agar: After one month at 20°C, the streak culture is yellowish-orange to orangish-red, the surface is smooth and shiny, and the margin is entire.

Dalmau plate culture on potato dextrose and morphology agars: After 7 days at 25°C, growth under the coverslip is hyaline and consists mostly of single budding cells. Well-developed pseudohyphae or true hyphae are not formed. In aged cultures (>3 weeks) in dilute (1:3) potato dextrose agar, elongated cells and short hyphal elements are sparsely produced.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+/w	Methanol	–
L-Sorbose	+/w	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	n
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	n
Soluble starch	–	DL-Lactate	+/w
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	w/–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	–

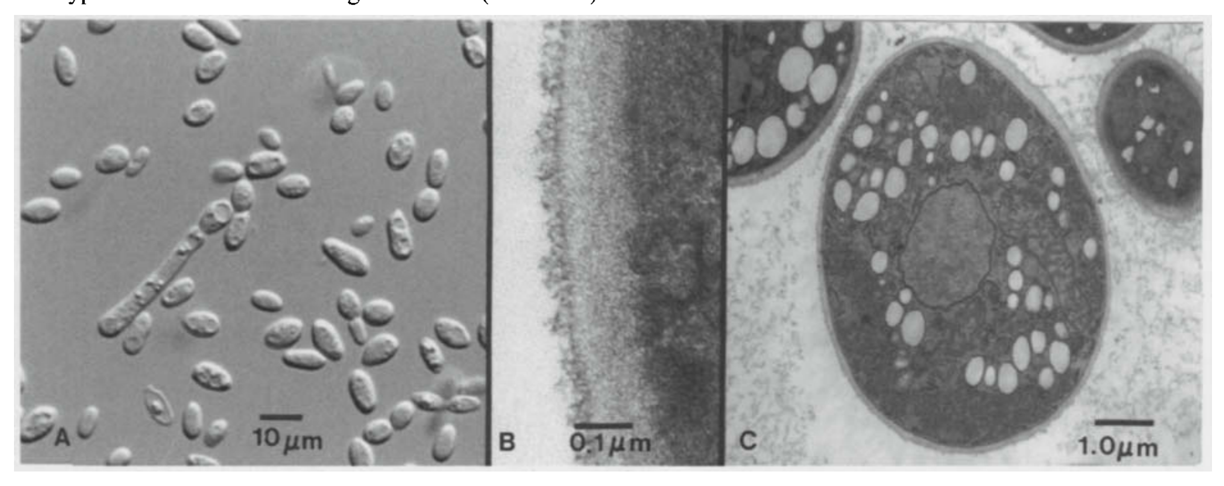


Fig. 285. A. Photomicrograph of vegetative cells of *S. complicata*; B. section of two-layered cell wall with slight capsule; C. whole cell showing nucleus and mitochondria.

Additional assimilation tests and other growth characteristics:

50% Glucose	–	Urease	+
Starch formation	–	Max. growth temp.	34°C

Co-Q: 10.

Mol% G+C: 51.5, IAM 12963 (holotype) (HPLC: Hamamoto et al. 1986b), 52.9 (Sugiyama et al. 1985); 50.9 IAM 12964 (CBS 7427, JCM 7359), HPLC: Hamamoto et al. 1986b).

Origin of strains studied: Soil from Laya, Bhutan; soil, Nepal (Goto and Sugiyama 1970).

Type strain: IAM 12963 (CBS 7301, JCM 7358) designated by Goto et al. (1987a).

Comments: *Saitoella complicata* was originally identified as a strain of *Rhodotorula glutinis* because both of these species have carotenoid pigmentation, lack fermentation, possess similar assimilatory spectra, and ascospores, ballistospores and teliospores were not observed. Subsequently, the two species were shown to differ in their cellular carbohydrate composition, ubiquinone systems, diazonium blue B color reactions (Sugiyama et al. 1985), DNA base composition (Hamamoto et al. 1987) and in their cell wall types (Goto et al. 1987a). Goto et al. (1987a) indicated similarities between *Saitoella* and the ascomycetous genera *Protomyces* and *Taphrina.* Analysis of DNA sequences from 18S rRNA coding regions for *T. wiesneri* and *S. complicata* indicated these species form an evolutionary lineage separate from the hemiascomycetes and euascomycetes (Nishida and Sugiyama 1993).

71. *Schizoblastosporion* Ciferri

M.Th. Smith

Diagnosis of the genus

Asexual reproduction is by bipolar budding in basipetal succession on a broad base.

Sexual reproduction is absent.

Fermentation is absent. Acetic acid is not produced. Nitrate is not assimilated. Diazonium blue B reaction is negative.

Type species

Schizoblastosporion starkeyi-henricii Ciferri

Species accepted

1. *Schizoblastosporion starkeyi-henricii* Ciferri (1930)

Systematic discussion of the species

71.1. *Schizoblastosporion starkeyi-henricii* Ciferri (1930a)

Growth on malt extract: After 3 days at 25°C, the cells are ellipsoidal, long-ellipsoidal or cylindroidal, (2.5–6.8)×(4.5–20) µm, single or in pairs. Buds are formed on a broad base at the poles (Fig. 286). A thin sediment is formed. After one month at 17°C, a sediment, a ring and, occasionally, some islets are present, but a pellicle is not formed.

Growth on malt agar: After one month at 17°C, the streak is grayish-brown, soft, dull, and flat.

Dalmau plate cultures on potato- and corn meal agars: Pseudomycelium is absent or consists of small chains of cells that sometimes have side branches.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	–
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Growth at 30°C	v
50% Glucose	–	Growth at 35°C	–
0.01% Cycloheximide	–		

Co-Q: Not determined.

Mol% G+C: 41.1–42.2, VKM Y-1947, VKM Y-1948 (Golubev and Vagabova 1977).

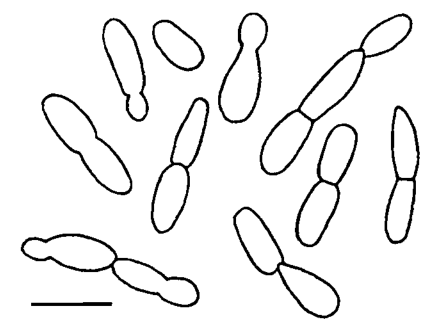


Fig. 286. *S. starkeyi-henricii*, CBS 2159. Bipolarly budding cells in malt extract after 3 days at 25°C. Bar = 10 µm.

Origin of the strains studied: CBS 2159, soil from peat bog, Lund; CBS 2914, forest soil, New Zealand, di Menna; CBS 4684, soil, Norway, Roberts; CBS 6297, soil, Germany, Windisch, and 3 additional strains isolated from soil, New Zealand, di Menna (1965b).

Type strain: CBS 2159, isolated by Lund.

Comments on the genus

The genus *Schizoblastosporion* exhibits bipolar budding in common with the genus *Kloeckera* and some species of the genus *Brettanomyces*, as well as with the teleomorphic genera *Hanseniaspora, Nadsonia, Saccharomycodes* and *Wickerhamia*. *Schizoblastosporion* can be distinguished from the latter genera by the absence of ascospores. It differs from *Kloeckera* by lack of fermentation and from *Brettanomyces* by absence of acetic acid production.

Schizoblastosporion chiloense Ramírez & González (1984j) is excluded from the present treatment because it shows multipolar budding and produces septate hyphae in addition to pseudomycelium. This species resembles *Dipodascus ingens* or *D. ovetensis*. Comparisons of LSU rDNA place *S. starkeyi-henricii* within the genus *Nadsonia* and *S. chiloense* near *Dipodascus ingens* (C.P. Kurtzman and C.J. Robnett, manuscript in preparation).

72. *Sympodiomyces* Fell & Statzell

A. Statzell-Tallman and J.W. Fell

Diagnosis of the genus

The streak culture on malt agar is dull cream, the surface is smooth, the texture soft but not mucoid. Yeastlike cells are present. A conidiophore develops directly from a yeast cell, producing a terminal conidium. Subsequent conidia are produced sympodially, i.e., at the side of the conidium a new growing tip of the conidiophore develops, forming a new terminal conidium resulting in an elongate conidiophore with successively formed conidia. As a result of conidial detachment the conidiophores possess several scars. Conidial formation is irregular, both in distance between conidia and location on the conidiophore. Branched true mycelium is inconspicuous and restricted in development. Conidia on the mycelium are single on a short peg or sympodial on an elongate conidiophore. Diazonium blue B, urease and fermentation tests are negative. Coenzyme Q-9 is present.

Type species

Sympodiomyces parvus Fell & Statzell

Species accepted

1. *Sympodiomyces parvus* Fell & Statzell (1971)

Systematic discussion of the species

72.1. *Sympodiomyces parvus* Fell & Statzell (1971)

Growth in 5% malt extract: After 5 days at 12°C, the single cells are polymorphic (Fig. 287); they are round, ovoid and subglobose, (1.0–1.3)×(1.3–3.4) μm. The cells are single, in pairs and short branched chains. The cells in chains are generally larger, (2.6–3.4)×(4.7–5.4) μm and ovoid in shape. The conidiophores at this age are short (which gives the appearance of typical budding yeast cells) or they are elongate to 4 μm. There is a moderate sediment. After one month, the conidiophores have elongated to 11 μm with numerous bud scars and one or more conidia.

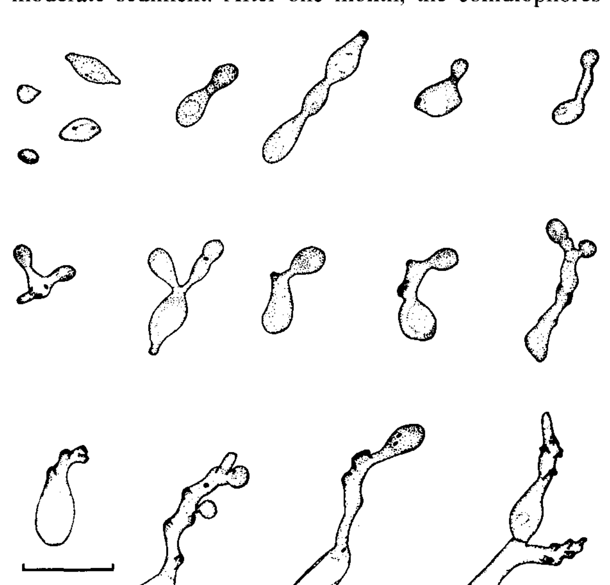


Fig. 287. *S. parvus*, CBS 6147. Yeast phase with the sympodial formation of conidia from culture grown for one to four weeks in malt extract at 12°C. Bar = 10 μm. *Camera lucida* drawings.

Growth on 5% malt agar: After one month at 12°C, the streak culture is dull cream, the surface is smooth, the texture is soft but not mucoid. The growth is convex and the periphery is entire, apart from tufts of non-aerial mycelium at the edges (Fig. 288).

Dalmau plate culture on corn meal agar: After one month at 12°C, there is true mycelium with distinct septa and clusters of conidia. The latter form in the same manner as single cells, i.e., the new bud forms terminally on a conidiophore that elongates sympodially to produce new conidia. The hyphae are approximately 2 μm in width; there are often spheroidal intercalary cells in the range of 5×7 μm. The mycelium formed is inconspicuous, and somewhat more extensive on malt agar than on corn meal agar.

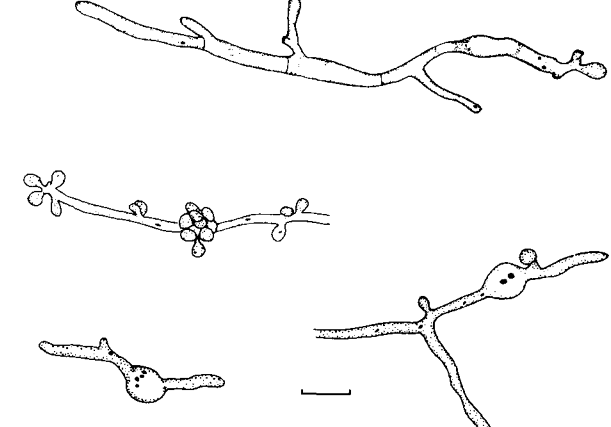


Fig. 288. *S. parvus*, CBS 6147. Hyphal formation after one week on malt agar at 12°C. Bar = 10 μm. *Camera lucida* drawings.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	s	Glycerol	+
Maltose	ws	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	s	Citrate	–
D-Arabinose	v	Inositol	s
D-Ribose	v	Hexadecane	–
L-Rhamnose	s	Nitrate	–
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Urease	–
5-Keto-D-gluconate	n	Gelatin liquefaction	–
Saccharate	–	Biotin-free	–
D-Glucuronate	s	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 25°C	+
10% NaCl/5% glucose	–	Growth at 30°C	–
Starch formation	–		

Co-Q: 9 (Sugiyama et al. 1991).

Mol% G+C: 46.3 (T_m: Sugiyama et al. 1991).

Cellular carbohydrate composition (mol%): glucose, 45.9; mannose, 40.6; galactose, 7.2; fucose, 4.2; ribose, 1.5; arabinose, 1.2 (Sugiyama et al. 1991).

Origin of the strains studied: 52 isolates from the open waters of the southern Indian and Pacific Oceans (Fell and Statzell 1971, Fell 1976).

Type strain: CBS 6147.

Comments on the genus

Sympodiomyces is an ascomycetous yeast; the diazonium blue B and urease test results are negative. Additionally, van der Walt and Hopsu-Havu (1976) and Kreger-van Rij (personal communication) report that the cell wall is typically ascomycetous. Sugiyama et al. (1991) state that the low mol% G+C content is typical of ascomycetous yeasts. The genus may be endemic to marine waters; the only reported occurrences (Fell and Statzell 1971, Fell 1976) have been from waters of Antarctic origin in the southern Pacific and Indian Oceans in the Antarctic Intermediate, Sub Antarctic, and South Pacific Central water masses from approximately 100°W westward to 118°E and 62°S northward to 42°S. Isolations were from depths of 16 to 748 meters where water temperatures ranged from 3.07–10.55°C and salinities were 33.94–34.85‰. Cell concentrations ranged from 1–5 per liter of sea water.

Information on the genus is limited. In addition to the original reports (Fell and Statzell 1971, Fell 1976), the genus has been studied by van der Walt and Hopsu-Havu (1976) who reported on the diazonium blue B reaction and cell wall structure and by Sugiyama et al. (1991) who compared the genus with *Sympodiomycopsis*. Kurtzman and Robnett (1995) demonstrated from comparisons of nucleotide sequences from the 5′ end of large subunit rDNAs that *Sympodiomyces, Blastobotrys, Arxula* and *Stephanoascus* are closely related and may be congeneric (Fig. 170, p. 385).

73. *Trigonopsis* Schachner

D. Yarrow

Diagnosis of the genus

Cells are triangular or ellipsoidal; tetrahedral and rhomboidal cells are sometimes present. Reproduction is by budding from the projecting angles on triangular and tetrahedral cells, and multilaterally on ellipsoidal cells. Neither hyphae nor pseudohyphae are formed.

Fermentation is absent. Nitrate is not assimilated. The cell wall is ascomycetous, comprising two layers. Diazonium blue B reaction is negative. Urea is not hydrolyzed.

Type species

Trigonopsis variabilis Schachner

Species accepted

1. *Trigonopsis variabilis* Schachner (1929)

Systematic discussion of the species

73.1. *Trigonopsis variabilis* Schachner (1929)

Growth on morphology agar: After 3 days at 25°C, the cells are ellipsoidal; triangular cells are usually also present, (3.5–6.0)×(2.0–6.0) μm, and they are single or in pairs (Fig. 289).

Growth on the surface of malt extract: After 1 month at 20°C, a ring and sometimes a pellicle, are present.

Slide culture on morphology agar: After 14 days at room temperature (20–22°C), there are neither hyphae nor pseudohyphae.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	s
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	s	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Biotin-free	–
D-Glucuronate	–	Pyridoxine-free	+
L-Lysine	v	0.01% Cycloheximide	+
D-Glucosamine (N)[1]	–	Growth at 37°C	+

[1] Utilization of D-glucosamine as a source of nitrogen.

Co-Q: Not determined.
Mol% G+C: 46.1, IFO 0755 (CBS 1040) (T_m: Nakase and Komagata 1971g).

Origin of strains studied: CBS 1040, beer in Germany; CBS 1041, variant of CBS 1040 which produces few triangular cells; CBS 4095, grapes in Italy.

Type strain: CBS 1040, "Rasse I" isolated from beer in Germany by Schachner (1929).

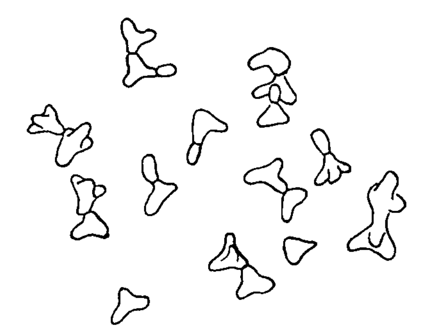


Fig. 289. *T. variabilis*, CBS 1041. After 3 days at 25°C in malt extract (Slooff 1970d).

Comments: The proportion of triangular cells to ellipsoidal cells formed by strains of this species is influenced by nitrogen source (Sentheshanmuganathan and Nickerson 1962, Picci and Verona 1962, Šašek and Becker 1969), carbon source (Matthewson and Barnett 1974), incubation temperature (Rambelli 1959, Picci and Verona 1962, Packer and Bersten 1977), presence of the detergents Tween 80 and Span 80, and of short-chain alcohols (Packer and Bersten 1977). It is also dependent on the strain, as is demonstrated by the variant CBS 1041.

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Part VIa

Classification of the basidiomycetous taxa

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Chapter 74

Discussion of teleomorphic and anamorphic genera of heterobasidiomycetous yeasts

T. Boekhout, R.J. Bandoni, J.W. Fell and K.J. Kwon-Chung

Contents

1. Introduction

Many heterobasidiomycetes have a unicellular budding haploid state alternating with a dikaryotic hyphal state, i.e., they are dimorphic and possess a yeast state. In some species, diploid, aneuploid or dikaryotic yeast states occur, and many anamorphic taxa are known only as yeasts. For teleomorphic yeasts, the hyphal state may be limited in culture, e.g., in some species of *Cystofilobasidium* Oberwinkler & Bandoni, but it can be indefinite and perhaps unlimited in many others, e.g., most species of *Rhodosporidium* Banno, *Tremella* Persoon and *Sirobasidium* de Lagerheim & Patouillard. With few exceptions, our knowledge of development in nature, ecology, and general biology of these fungi is fragmentary or nonexistent. Species parasitizing economically important annual plants (e.g., Ustilaginales) have hyphal phases of known duration and extent, but even in these species, little is known of the frequency of occurrence or distribution of the free-living yeast states. For some taxa, e.g., the sporobolomycetaceous taxa, the abundant and widespread occurrence of the yeast states is known, but occurrence of dikaryotic mycelia in nature is essentially unknown.

The terms "yeast" or "yeast state" are applied to the budding unicellular phase or phases in heterobasidiomycetous life histories. These yeast states are readily obtained from basidiospores in species with macroscopically visible basidiomata, e.g., many species of *Tremella, Sirobasidium, Holtermannia* Saccardo & Traverso, *Cystobasidium* (de Lagerheim) Neuhoff, *Mycogloea* Olive, and from those causing visible disease symptoms in plants, e.g., species of *Ustilago* (Persoon) Roussel, *Itersonilia* Derx, and others. The yeast states provide one approach to studies of relationships of budding fungi, including determining relationships of anamorphic yeasts of uncertain affinity. Yeasts or yeast states are presently known from Agaricostilbales, Atractiellales, Cryptobasidiales, Exobasidiales, Filobasidiales, Graphiolales, Platygloeales, Septobasidiales, Sporidiales, Tremellales, and Ustilaginales (Oberwinkler 1987, and see below). However, yeast states of many of these groups of organisms are rarely isolated in nature, i.e., other than from the basidiocarps.

Fungi that form short hyphal fragments or cellular aggregates are frequently referred to as yeastlike, e.g., species of *Trichosporon* Behrend. It should be stressed, however, that the distinctions among the various morphs (unicellular, yeastlike and pseudohyphal) are not always clear. Yeast/hyphal dimorphism which occurs in many groups of Heterobasidiomycetes, seems to be an important taxonomic feature. This monograph includes only a small number of those taxa in which obligatory and stable yeast states occur in the normal course of the life history.

2. Taxonomy of heterobasidiomycetous yeasts

Brefeld (1881, 1888, 1895a,b) was the first mycologist to make detailed observations of basidiospore germination in heterobasidiomycetous fungi. He described and illustrated budding growth in cultures of many smuts and jelly fungi, and he utilized dimorphism as an important character in his classification. Brefeld's student, A. Möller, followed his example in studies of Brazilian jelly fungi (Möller 1895). Although Brefeld's ideas on dimorphism received little support over the next century, some aspects of his classification system did survive.

The taxon "Heterobasidiomycetes" originated with Patouillard (1900), and the term has been applied at some level (i.e., class, subclass) since that time. The Heterobasidiomycetes have encompassed taxa with septate or deeply divided basidia, complex life histories, basidia often arising from distinct probasidia (teliospores or equivalents in rusts, smuts and others), and germination of basidiospores by ballistoconidia or conidia of other types as opposed to Homobasidiomycetes. Modified versions of this classification system have been in use until the present, by a variety of authors, e.g., Martin (1945, 1952), Lowy (1971), Jülich (1981), and Locquin (1984). Bessey (1950) recognized three subclasses of Basidiomycetes: 1. Teliosporae (fungi with teliospores and transversely septate basidia, and including rusts, smuts, and related fungi), 2. Heterobasidiae (often with gelatinous basidiomata and septate basidia, teliospores or equivalents lacking in most taxa, and including Tremellales, Auriculariales, Dacrymycetales, and Tulasnellales),

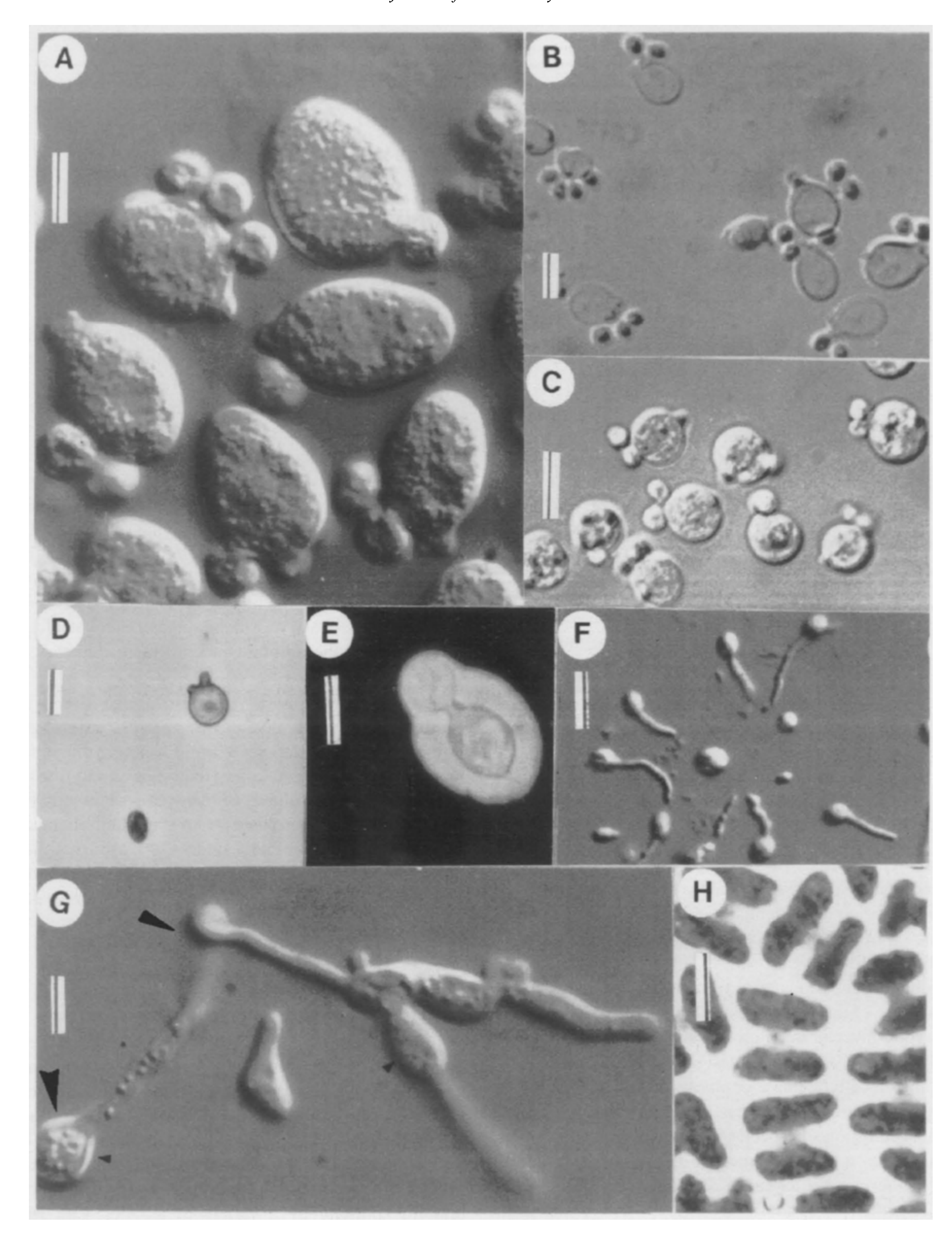


Fig. 290. A–C. Basidiospore germination in three species of *Tremella.* (A) Germinating basidiospores of *T. mesenterica,* in which the primary buds remain attached to the basidiospores and produce deciduous buds. Bar = 5 µm. (B) *T. fuciformis,* RJB 7668, China. Bar = 5 µm. (C) *T. globispora* Reid, RJB 7883, Canada. Bar = 10 µm. (D)–(F) *T. mesenterica* yeast cells. (D) Cell with two adjacent budding loci, the length of which suggests numerous buds have been produced, RJB 2259, Canada. Bar = 5 µm. (E) Budding cell mounted in India ink to show thick capsule, RJB 2259. Bar = 5 µm. (F) Mixed compatible cells, most of which have developed conjugation tubes, RJB 2259 Bar = 10 µm. (G) *T. mesenterica* dikaryotic hypha with two clamps, developed after conjugation of two cells (indicated by arrows), RJB 2285. Bar = 5 µm. (H) *Trimorphomyces papilionaceus,* dikaryotic cells of the form produced both as conidia from filamentous conidiogenous cells and by budding of these, or of the indistinguishable H-shaped yeast cells, RJB 6646 Type, Canada. Bar = 5 µm.

and 3. Eubasidiae for the Homobasidiomycetes (basidia aseptate and not deeply divided).

Talbot (1968) also used three classes, Teliomycetes, Phragmobasidiomycetes, and Holobasidiomycetes, discarding the name Heterobasidiomycetes. Talbot considered the Dacrymycetales and Tulasnellales to be Holobasidiomycetes, and he did not emphasize dimorphism in his classification scheme.

Patouillard's classification permitted ready identification of families, since these were largely based upon basidial morphology. Although the Heterobasidiomycetes and Homobasidiomycetes differed in the types of basidiospore germination, yeast states were not emphasized in Patouillard's classification system. Budding of basidiospores was often mentioned in descriptions, but dimorphism was essentially unknown and unstudied in most basidiomycetous groups until the middle of the 20th century. The smuts are an exceptional group among the Heterobasidiomycetes in that their yeast states were used experimentally to examine mating genetics. Since mating initiates the parasitic phase and determines virulence, there were numerous reports on teliospore germination and mating of "sporidia" in the group (see Fischer and Holton 1957, for extensive references). The first attempt to demonstrate mating pheromones in true fungi was carried out with sporidia of *Ustilago* spp. (Bauch 1925).

It is clear that dimorphic fungi formerly placed in the Heterobasidiomycetes do not form a natural group, but the name is convenient to use as an informal category in studies of dimorphic basidiomycetes. Heterobasidiomycetous yeasts, like ascomycetous yeasts, bud to form soft, often slimy or mucoid colonies. The yeast state usually commences with basidiospore germination (e.g., as in Fig. 290) or cells are budded directly from basidia, as in species of *Agaricostilbum* and *Rhodosporidium* (Fig. 291). This unicellular haploid state ends at the initiation of the dikaryotic state in those yeasts with known teleomorphs. The majority of Heterobasidiomycetes with haploid yeast states have dikaryotic mycelia that are parasitic on green plants or fungi.

As noted above, budding basidiospores of *Tremella* and *Sirobasidium* were observed and illustrated in the nineteenth century (Brefeld 1881, 1888, Möller 1895). The presence of ballistoconidia in *Sporobolomyces* Kluyver & van Niel led Kluyver and van Niel (1924, 1927) to suggest that this yeast is basidiomycetous, based on the morphological resemblance of ballistoconidia with actively discharged apiculate basidiospores. The discovery of mating and a sexual state in strains of *Rhodotorula glutinis* (Fresenius) Harrison by Banno (1963, 1967), and the subsequent discovery of sexual states in species of *Rhodosporidium* (Fig. 291) and *Leucosporidium* (Fell 1970b, 1974, Fell et al. 1969, 1973), *Sporidiobolus* Nyland (Nyland 1949, Bandoni et al. 1971, 1975, Fell and Statzell-Tallman 1980b, 1981, 1982), *Filobasidium* Olive (Olive 1968, Rodrigues de Miranda 1972, Kwon-Chung 1977b, Bandoni et al. 1991), *Filobasidiella* Kwon-Chung (Kwon-Chung 1975), *Cystofilobasidium* (Oberwinkler et al. 1983), and *Bulleromyces* Boekhout et al. (Boekhout et al. 1991a, demonstrated the basidiomycetous nature of these organisms.

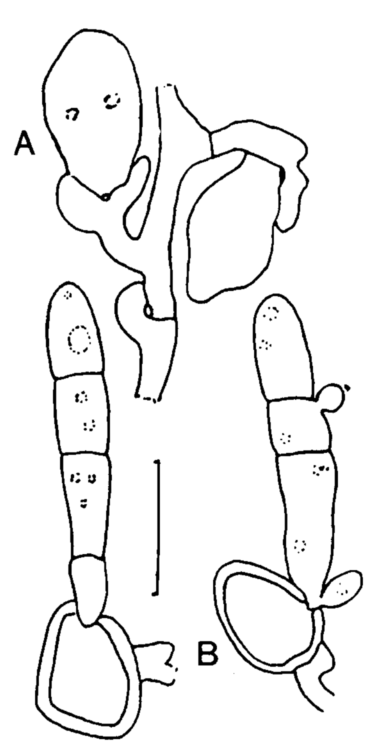


Fig. 291. *Rhodosporidium toruloides* Banno (Sporidiales). (A) Mature teliospore. (B) Germinating teliospore with basidia. Bar = 10 μm. (Teliospores obtained by crossing IFO strains 10034 and 0539_2. Strains provided courtesy of Dr. I. Banno.)

Donk (1973a–c) proposed placement of the basidiomycetous yeasts in Brefeld's hemibasidiomycetes without assigning them to orders. Donk's concept of hemibasidiomycetes included Ustilaginales, Tilletiales, and Graphiolales.

In the previous edition of this book the basidiomycetous yeasts were divided into five groups: 1. Sporobolomycetaceae Derx (both anamorphic and teleomorphic ballistosporous yeasts), 2. Cryptococcaceae Kützing (the remaining anamorphic basidiomycetous yeasts), 3. Filobasidiaceae L. Olive (teleomorphic yeasts with holobasidia), 4. teliospore-forming yeasts (teleomorphic taxa with teliospores) and 5. Tremellales (yeast stages of genera belonging to the Tremellales). Van der Walt (1987) made a clear distinction between teleomorphic and anamorphic basidiomycetous yeasts, which is reflected in his taxonomic concepts (see below). He placed anamorphic yeasts in either the Cryptococcaceae Kützing emend. van der Walt (1987) or the Sporobolomycetaceae Derx emend. van der Walt (1987).

Basidial morphology is considered an important character for the affiliation of basidiomycetous yeast genera to higher taxa. Transversely septate basidia commonly occur in yeasts belonging to the Sporidiales, e.g., *Leucosporidium, Rhodosporidium* and *Sporidiobolus* (Fell

1984a). However, some of these yeasts have one-celled basidia as well, e.g., *Sporidiobolus johnsonii* (Nyland 1949, Bandoni et al. 1971, Fell and Statzell-Tallman 1981). Cruciately septate basidia are characteristic for most Tremellales (Bandoni 1987, Oberwinkler 1987), and occur in *Bulleromyes,* the teleomorph of *Bullera alba* (Boekhout et al. 1991a). Holobasidia occur in species belonging to the Filobasidiales, e.g., *Filobasidiella* (Kwon-Chung 1975, 1987), *Filobasidium* (Olive 1968), *Cystofilobasidium* (Oberwinkler et al. 1983, Hamamoto et al. 1988b), and *Xanthophyllomyces* Golubev (Golubev 1995). Teliospores (thick-walled probasidia) commonly occur in the Sporidiales, but have also been found in filobasidiaceous yeasts, such as *Cystofilobasidium* and *Mrakia* (Oberwinkler et al. 1983, Kwon-Chung 1987, Yamada and Komagata 1987). Haustorial branches seem to indicate a Tremellales–Filobasidiales relationship, but the haustorial structures of some simple-pored mycoparasites may be functionally similar (Oberwinkler et al. 1990a).

Anamorphic yeasts can be assigned to the Basidiomycetes by a number of morphological, biochemical, ultrastructural, and physiological criteria. Basidiomycetous yeasts have a positive Diazonium blue B reaction (Simmons and Ahearn 1987, van der Walt and Hopsu-Havu 1976, Hagler and Ahearn 1981), contain pyrophosphatidic acid (Goto et al. 1988), and in contrast with most ascomycetous yeasts, show urease activity (Abadie 1961, 1967, Roberts et al. 1978, Booth and Vishniac 1987). The presence of ballistoconidia and/or red carotenoid pigments also indicate a basidiomycetous affinity. Other basidiomycetous characteristics are found in the ultrastructure of the cell wall (Kreger-van Rij and Veenhuis 1971a, Simmons and Ahearn 1987, Boekhout et al. 1992b) and septa (e.g., Johnson-Reid and Moore 1972, Khan and Kimbrough 1980, Kreger-van Rij and Veenhuis 1971a,b, Moore and Kreger-van Rij 1972, Kwon-Chung and Popkin 1976, Moore 1978, 1979, 1987b, Boekhout et al. 1992b), and in the biochemical composition of the cell wall and capsules (Sugiyama et al. 1985, Weijman and Golubev 1987, Weijman and Rodrigues de Miranda 1983, 1988, Suzuki and Nakase 1988a, Prillinger et al. 1993).

In contrast to most ascomycetous yeasts, the mol% G + C of the majority of basidiomycetous yeasts is above 50 (von Arx 1979b). The coenzyme Q systems are mainly Co-Q 9 and Co-Q 10 (Kuraishi et al. 1985), but lower numbers of isoprenologs occur. More recently, killer toxin (mycocin) spectra have been used to characterize basidiomycetous yeasts. Mycocins of basidiomycetous origin usually act only against other basidiomycetous yeasts (e.g., Golubev 1989a, 1991b, Golubev and Kuznetsova 1989, Golubev and Tsiomenko 1985, Golubev et al. 1988). In summary, basidiomycetous yeasts are unicellular fungi belonging to the Heterobasidiomycetes, characterized by the presence of multilamellate electron-dense cell walls, the presence of xylose and/or fucose in whole-cell hydrolyzates, and the presence of dolipores, or diaphragm-like pores without Woronin bodies ("simple" pores), or micropore-like structures.

3. Molecular phylogeny

Early molecular studies of 5S ribosomal RNA suggested the presence of two main lines of phylogenetic divergence among basidiomycetous yeasts, which correlates with septal ultrastructure (Walker and Doolittle 1982, Templeton 1983). In more extensive work, including a larger number of heterobasidiomycetous species, the yeast and yeastlike species were shown to belong to four out of eight clusters that correlate well with septal morphology (Walker 1984). This has largely been supported by Blanz and Gottschalk (1984). Two main clusters, one containing Sporidiales and their anamorphs and the other tremellaceous and filobasidiaceous species, can be distinguished from the analysis of partial 26S rRNA and from entire 18S rRNA/rDNA sequences (Fell et al. 1992, 1995, Van de Peer et al. 1992, Suh and Sugiyama 1993b, Sugiyama and Suh 1993, Swann and Taylor 1993, 1995a). This clustering correlates with septal ultrastructure (dolipore versus "simple" pore) and cell wall biochemistry (presence or absence of xylose).

A comparison of 26S rDNA partial sequences from 145 basidiomycetous yeast species (Fell et al. 1995) demonstrated four main clusters: Sporidiales (nom. nud.), Tremellales, *Cystofilobasidium* and related taxa, and the Ustilaginales. Within the Sporidiales there was a major cluster that included *Sporidiobolus* spp., *Rhodosporidium toruloides* and related anamorphic and teleomorphic species. Other subclusters demonstrated molecular divergence among the anamorphic and teleomorphic genera *Bensingtonia, Rhodotorula, Sporobolomyces, Kurtzmanomyces, Sterigmatomyces, Erythrobasidium, Leucosporidium* and *Rhodosporidium.* The *Cystofilobasidium* cluster included a diversity of genera: *Cystofilobasidium* spp., *Mrakia, Trichosporon pullulans, Itersonilia perplexans, Phaffia* and *Cryptococcus aquaticus.* The Tremellales demonstrated a similar diversity. The genus *Trichosporon,* with the exception of *T. pullulans,* remained a monophyletic clade as did the separate clades *Filobasidiella, Filobasidium* and *Fellomyces.* The genus *Cryptococcus* was found to be polyphyletic, and occurred in several subclusters. Other genera occurring in the Tremellales cluster were *Sirobasidium, Apiotrichum, Holtermannia, Tremella* and *Bulleromyces.* The limited study of the Ustilaginales cluster indicated a similar polyphyletic nature, and included the genera *Entyloma, Pseudozyma, Tilletia, Tilletiaria, Tilletiopsis* and *Ustilago.*

In a comparison of partial 18S rDNA sequences of basidiomycetous yeasts with entire 18S rDNA sequences of several representatives of Basidiomycetes, the yeasts were found to belong to the Tremellomycetidae and the Urediniomycetes, respectively (Swann and Taylor 1995a). Within this latter group, the yeasts belonged to three clades, namely 1. *Agaricostilbum* clade (*Bensingtonia yuccicola*), 2. Sporidiales clade (*Sporobolomyces*

roseus, Leucosporidium scottii, Rhodosporidium toruloides, Rhodotorula glutinis, and 3. *Erythrobasidium* clade (*Erythrobasidium hasegawianum*). The detailed phylogenetic relationships among Heterobasidiomycetes with or without yeast states require further biochemical, ultrastructural and molecular investigations.

4. Characterization of orders and families

4.1. Anamorphic basidiomycetous yeasts

The anamorphic basidiomycetous yeasts are currently assigned to two families:

4.1.1. Cryptococcaceae Kützing (1833) emend. van der Walt (1987): The family is restricted to anamorphic heterobasidiomycetous yeasts and yeastlike fungi; budding is enteroblastic; cell walls are lamellate and hyphal septa have dolipores if perforate; parenthesomes may be present; cell hydrolyzates contain xylose. The type genus is *Cryptococcus.* Other genera are *Bullera, Fellomyces, Kockovaella, Phaffia, Trichosporon, Tschuchiyaea, Udeniomyces* and perhaps *Hyalodendron* and *Moniliella.* These genera usually assimilate *myo*-inositol and D-glucuronate and produce extracellular starch-like compounds. It can be foreseen that generic concepts will change in the near future as a consequence of the widely divergent ribosomal nucleotide sequences observed in several genera, e.g., *Cryptococcus* and *Bullera* (Guého et al. 1993, Fell et al. 1992, 1995, Nakase et al. 1993). In contrast, other genera, e.g., *Trichosporon,* with the exclusion of *T. pullulans,* were found to be relatively homogeneous (Guého et al. 1992b).

4.1.2. Sporobolomycetaceae Derx (1948) emend. van der Walt (1987): The family is restricted to anamorphic yeasts and yeastlike fungi; budding is enteroblastic; cell walls are lamellate and hyphal septa are diaphragm-like ("simple") if perforate; cell walls are without xylose, but fucose, rhamnose and galactose may be present. The type genus is *Sporobolomyces.* Other genera are *Bensingtonia, Kurtzmanomyces, Sterigmatomyces* and *Rhodotorula.* These genera usually do not assimilate *myo*-inositol and D-glucuronate, and do not form extracellular starch-like compounds. Sequence divergence of ribosomal RNA (rRNA) or rDNA suggests taxonomic heterogenity for a number of genera, such as *Bensingtonia* and *Sporobolomyces* (Nakase et al. 1993, Fell et al. 1992, 1995).

These anamorphic families are artificial taxa, which are maintained here for convenience. It can be expected that they will become superfluous when the taxonomy of the anamorphic basidiomycetous yeasts becomes integrated with the teleomorphic Heterobasidiomycetes. The two anamorph families are partly in accord with the Rhodotorulaceae Lodder (1934). However, Lodder's family concept was based on the presence of carotenoid pigments. Recent molecular, biochemical and ultrastructural studies indicate the Rhodotorulaceae is a paraphyletic group.

4.2. Teleomorphic Heterobasidiomycetes with yeast states

The Heterobasidiomycetes with yeasts and yeastlike-states are distinguished by basidial morphology, septal pore anatomy, and cell wall composition. The classification of families and orders rests largely on basidial morphology. However, similar basidial types may be present in categories with different septal pores, e.g., the cruciately septate basidium occurs in the Tremellales and Auriculariales. Also, the cylindric, transversely septate basidium occurs in such families and orders as the Auriculariaceae, Platygloeales, Uredinales, Septobasidiales, Ustilaginales, Sporidiales and Tremellales. Presently, the pore structure, which correlates well with molecular data (Swann and Taylor 1993), appears to be a more definitive character.

4.2.1. Sporidiales R.T. Moore (nomen nudum, no Latin diagnosis): This order contains the so-called "saprobic smuts". Nyland (1949) placed *Sporidiobolus* among the Heterobasidiomycetes. Other teliospore-forming fungi, specifically the rusts and smuts were placed in a separate class, Teliomycetes (Ainsworth 1973) or subclass Teliomycetidae (Alexopoulos and Mims 1979). The life-cycles of the teliospore-forming yeasts are similar to those of the smuts (see, e.g., Banno 1967, Fell 1984a, Fell et al. 1969). Johnson-Reid and Moore (1972) interpreted the teliospores as encysted proasci and named them ustospores. Moore (1972) placed the ustospore-forming fungi in a separate division Ustomycota (Moore 1972, 1980), characterized by the presence of ustospores and attenuated diaphragm-like ("simple") septa lacking a parenthesome. The Ustomycota were subdivided in the Ustomycetes (smuts) and Sporidiomycetes (yeasts). The latter contained the order Sporidiales, in which two families have been distinguished to accommodate the yeasts and yeastlike fungi, viz., Sporidiaceae R.T. Moore and Sporidiobolaceae R.T. Moore for non-ballistosporous and ballistosporous teliospore-forming yeasts, respectively (Moore 1980). Recent molecular studies indicate that the Ustomycota are polyphyletic (Swann and Taylor 1993, 1995a).

At present, it is appropriate to maintain the teliospore-forming, and phylogenetically related yeasts, in a separate order. We consider the Sporidiaceae and Sporidiobolaceae to be synonymous because they cannot be discriminated by rRNA/rDNA sequence analysis, septal ultrastructure and cell wall biochemistry. We choose Sporidiobolaceae R.T. Moore, with *Sporidiobolus* Nyland as type, which was the first genus to be described in this family (Nyland 1949).

4.2.1.1. Sporidiobolaceae R.T. Moore (1980) emend. (synonym Sporidiaceae R.T. Moore): This familiy is restricted to teleomorphic yeasts and yeastlike fungi; budding is enteroblastic; dikaryotic mycelium with or without clamp connections, haustorial branches are absent; somewhat thick-walled teliospores are present, basidia are usually transversely septate, but occasionally one-celled; cell walls are lamellate and hyphal septa have

attenuated diaphragm-like ("simple") septa if perforate and without Woronin bodies; no dolipore is present; xylose is absent in whole-cell hydrolyzates, but fucose, galactose and/or rhamnose may be present. The type genus is *Sporidiobolus*, and other assigned genera are *Leucosporidium* and *Rhodosporidium*.

Sporidiobolus, a ballistoconidia-forming, teliospore-forming, and carotenoid pigments producing genus (Nyland 1949), was proven to be the teleomorph of the red-pigmented yeast species of *Sporobolomyces* (Bandoni et al. 1971, 1975, Fell and Statzell-Tallman 1981, Boekhout 1991a). Banno (1963, 1967) described the genus *Rhodosporidium*, and Fell et al. (1969) published *Leucosporidium*, the teleomorph genus for the non-carotenoid species *Candida scottii*.

The life cycles include homothallic and heterothallic systems, that may occur within a single species. In the heterothallic system, compatible mating types conjugate and develop a dikaryotic mycelium with clamp connections. Karyogamy takes place in the teliospore, which germinates to a one- to four-celled basidium where meiosis occurs. The heterothallic incompatibility systems are biallelic unifactorial (bipolar), multiple allelic unifactorial or bifactorial (tetrapolar). The homothallic lifecycle is distinguished by dikaryotic hyphal cells that develop from a diploid cell in the absence of mating. Clamp connections may be present, and karyogamy apparently takes place in the teliospore. In *Sporidiobolus johnsonii* and *S. ruineniae*, meiosis does not seem to occur in the basidium, which results in diploid basidiospores. In some cases, e.g., in homothallic strains of *Rhodosporidium toruloides*, the diploid cells appear to originate from a failure during meiosis. The progeny from homothallic *Rhodosporidium toruloides* are either homothallic or heterothallic. Quantitation of the amount of nuclear DNA by fluorescence cytometry indicates that self-sporulating strains of *Leucosporidium scottii* are diploid (Suh et al. 1993b). Strains of several yeasts, e.g., *Leucosporidium scottii* and *Sporidiobolus pararoseus*, produce teliospores in the absence of mating. Rather than the previously discussed life cycles, the hyphal cells remain uninucleate and clamp connections are lacking. Here, teliospore formation seems the result of monokaryotic fruiting (Fell 1984a). In addition to monokaryotic fruiting, these strains may restore the heterothallic cycle by mating with a compatible strain.

The genera *Leucosporidium*, *Rhodosporidium* and *Sporidiobolus* were found to be phylogenetically related to smuts that occur on dicotyledonous hosts (Blanz and Gottschalk 1984). 18S rRNA gene sequences placed *Sporidiobolus johnsonii* and *Leucosporidium scottii* in the "simple-septate" lineage, together with *Cronartium ribicola* (Swann and Taylor 1993). Analysis of 18S and 26S rRNA sequences of teliospore-forming yeasts support the transfer of some species formerly classified in *Rhodosporidium* and *Leucosporidium* to *Cystofilobasidium*, e.g., *C. infirmominiatum*, *C. bisporidii*, *C. lari-marini*, *C. capitatum*, and *Mrakia*, e.g., *M. gelidum*, *M. nivalis*, *M. frigidum* and *M. stokesii* (Hamamoto et al. 1988b, Fell and Statzell-Tallman 1992, Yamada and Kawasaki 1989a, Suh and Sugiyama 1993b). Some other taxonomic changes based on nucleotide analysis have been proposed, namely *Kondoa* and *Sakaguchia*, accommodating *Rhodosporidium malvinellum* and *Rhodosporidium dacryoidum*, respectively (Yamada et al. 1989a, 1994a). *Kondoa* was found to belong to the *Agaricostilbum* clade, and *Sakaguchia* (Yamada et al. 1994a) belongs to the *Erythrobasidium* clade (Swann and Taylor 1995a). Based on partial 26S rRNA sequences, species of *Sporidiobolus*, *Leucosporidium* and *Rhodosporidium* form distinct subclusters among the Sporidiobolaceae, together with anamorphic species belonging to *Bensingtonia*, *Rhodotorula* and *Sporobolomyces* (Fell et al. 1992, 1995). The close phylogenetic relationship among ballistoconidia and non-ballistoconidia-forming species, e.g., *Sporidiobolus microsporus* and *Rhodosporidium fluviale*, questions the use of ballistocondia as a useful phylogenetic characteristic among basidiomycetous yeasts.

Erythrobasidium hasegawae, which lacks teliospores, has some phenetic characters in common with the teliospore-forming yeasts (Hamamoto et al. 1988c, Suh and Sugiyama 1993b, Sugiyama and Suh 1993), but was found to belong to a separate clade (Swann and Taylor 1995a). Molecular analysis suggests that *Heterogastridium pycnidioideum* Oberwinkler & Bauer, a teleomorphic heterobasidiomycete with pycnidioid basidiocarps, "simple" septa, and with fucose, but without xylose in the cell wall (H.J. Roeijmans, unpublished observations), and thus far classified in the Heterogastridiales (Oberwinkler et al. 1990b), belongs to the Sporidiales clade (Swann and Taylor 1995a).

4.2.2. Ustilaginales and Tilletiales: Although the economically important smut and bunt fungi have been studied more intensively than many other Heterobasidiomycetes, their classification and relationships remain unclear. Two orders, Ustilaginales and Tilletiales, are often recognized, but they are not generally accepted. Alternatively, when a single order Ustilaginales is used, it is difficult to assign many smut taxa to the families Ustilaginaceae and Tilletiaceae (L.R. Tulasne and C. Tulasne 1847). The Ustilaginales and Tilletiales differ in several important aspects, among which are the basidia, septal pore anatomy, haploid states and conidial types, which support classification in two orders. However, molecular phylogenetic studies suggest a relatively close relationship (Swann and Taylor 1993). Some common genera in temperate areas include *Ustilago*, *Microbotryum*, *Cintractia*, *Schroeteria* and *Sphacelotheca*.

Ustilago is the largest genus in the order with approximately 300 species, and most species parasitize monocotyledonous hosts. Teliospores of *Ustilago* spp. typically germinate with a cylindrical, four-celled basidium. Each basidial cell repeatedly buds off basidiospores (sporidia),

which produce the haploid yeast state. Teliospore germination readily occurs in many species, or it can be induced by treatments such as soaking in water or plant extracts (Fischer and Holton 1957). Conjugation can often be seen when compatible cell types are mixed, but the dikaryophase may be unstable in culture and few species have been grown through the complete life history. Basidium maturation in several species occurs without sporidial development, the four basidial cells conjugating directly in two pairs. Yeast states develop in these species only infrequently when one or more basidial cells remain unconjugated.

Species of *Leucosporidium, Rhodosporidium* and *Sporidiobolus* have a life cycle similar to *Ustilago* species, but are currently classified in the Sporidiales (see above). Yeastlike anamorphs of Ustilaginales classified in various genera, viz., *Candida fusiformata, C. tsukubaënsis, Pseudozyma prolifica, Sporobolomyces antarcticus, "Stephanoascus" flocculosus, "S." rugulosus, Sterigmatomyces aphidis* and *Trichosporon oryzae* have been reclassified in *Pseudozyma* Bandoni emend. Boekhout (Boekhout 1995). Molecular analysis indicates that these taxa form a monophyletic group with *Ustilago maydis* (Boekhout et al. 1995a, Fell et al. 1995).

Tilletia, with about 70 species that mainly parasitize grasses, has a cylindric basidium that is aseptate or sometimes adventitiously septate and bears elongate basidiospores apically. Only one spore per locus is formed. The basidiospores often conjugate before release, in which case the H-shaped conjugants give rise directly to dikaryotic mycelial colonies or, alternatively, conjugation occurs between closely situated compatible pairs after their release. Ballistoconidia often develop on the mycelia. They are produced and abstricted in the manner of basidiospores of most basidiomycetes. The septal pores are often more complex than those of the Ustilaginales, but they do not reach the complexity of dolipores.

The anamorphic ballistoconidia-forming genus *Tilletiopsis* was placed in the Filobasidiaceae by von Arx et al. (1977). This concept is not supported by septal ultrastructure and cell wall biochemistry (Boekhout 1987, Boekhout et al. 1992b). Morphological, biochemical and molecular similarities suggest *Tilletiopsis* to be related to *Tilletiaria,* a teliospore- and ballistoconidia-forming genus (Bandoni and Johri 1972, Boekhout et al. 1995a). The hyphal system and the presence of falcate ballistoconidia in these genera strongly resembles that of the Tilletiales (Boekhout et al. 1992b). Placement of *Tilletiaria* in the Tilletiariaceae Moore (1980) seems appropriate. Budding is unknown or is limited in the Tilletiales, and occurs, e.g., in *Tilletiaria anomala* (Tilletiariaceae, Tilletiales) and *Tilletiopsis washingtonensis* (Boekhout 1991a).

4.2.3. Graphiolales: This order contains a small number of poorly-known species of which only the widely spread *Graphiola phoenicis* has been studied in detail. The fungus produces minute blister-like sori on palm leaves. Catenate globose cells within the sorus are simple basidia. Meiosis occurs in these cells, and haploid basidiospores bud directly from the basidia to yield yeast colonies. The morphology is unlike that of any other basidiomycete, but it is conceivable that the chains of basidia are homologues of chains of teliospores in the Ustilaginales and of basidial chains in the Sirobasidiaceae.

4.2.4. Cryptobasidiales: Although they are holobasidiate, these fungi resemble smuts in their life histories. Basidia are found in minute clusters on the infected leaves of Laurales and Rhamnaceae in the case of *Coniodyctum* species, or of Fagaceae and Juglandaceae for species of *Microstroma.* The basidiospores, borne on clavate basidia, are not forcibly abstricted, and they give rise to yeast colonies in *Coniodyctum chevalieri* and in *Microstroma juglandis.* Other species in these genera have not been studied in detail.

4.2.5. Exobasidiales: This order includes relatively few described species (<100 spp.) and, with the exception of some *Exobasidium* spp., few are well known. The Exobasidiales, including ca. seven genera in two families, are all plant parasites and found on a variety of plant species. The dikaryotic mycelium grows internally in the host (mostly on leaves of Vacciniaceae in *Exobasidium*), transforming leaves into hypertrophied, inflated, bladdery structures suggestive of fruits. The fine hyphae have "simple" septal pores and they produce basidia at the surfaces of infected parts. The clavate, non-septate basidia, have stout sterigmata whose curvatures are opposite to those in other basidiomycetes. The basidiospores become septate and germinate by producing conidia successively on narrow outgrowths. Haploid states are unicellular yeasts in some species, but the colonies of others consist of short hyphal segments and are more appropriately described as yeastlike.

Exobasidiellum gramineum infects grass leaves; the affected areas are without the hypertrophy associated with *Exobasidium* infections. The basidia and basidiospores are similar to those of *Exobasidium* spp.

Exobasidium spp. and *Laurobasidium lauri* (Geyler) Jülich apparently have similar cell wall carbohydrate compositions (Prillinger et al. 1990b, Prillinger et al. 1993). *L. lauri,* is an unusual species inducing clavarioid galls on *Laurus azorica* in Europe. In addition to the unusual galls, *L. lauri* has clamp connections, which are absent in *Exobasidium.*

Dicellomyces gloeosporus produces gelatinous tuberculate to discoid basidiomata on the leaf surfaces of canes of *Arundinaria* spp. in the southern USA. *Brachybasidium pinangae* also produces pustulate basidiomata, but on *Pinanga* spp. (Palmae) from Java. The bisterigmate basidia have slightly thickened probasidial walls. These two genera are sometimes put in a separate order, Brachybasidiales (e.g., McNabb and Talbot 1973), but their features are rather close to those of other Exobasidiales (Oberwinkler 1987).

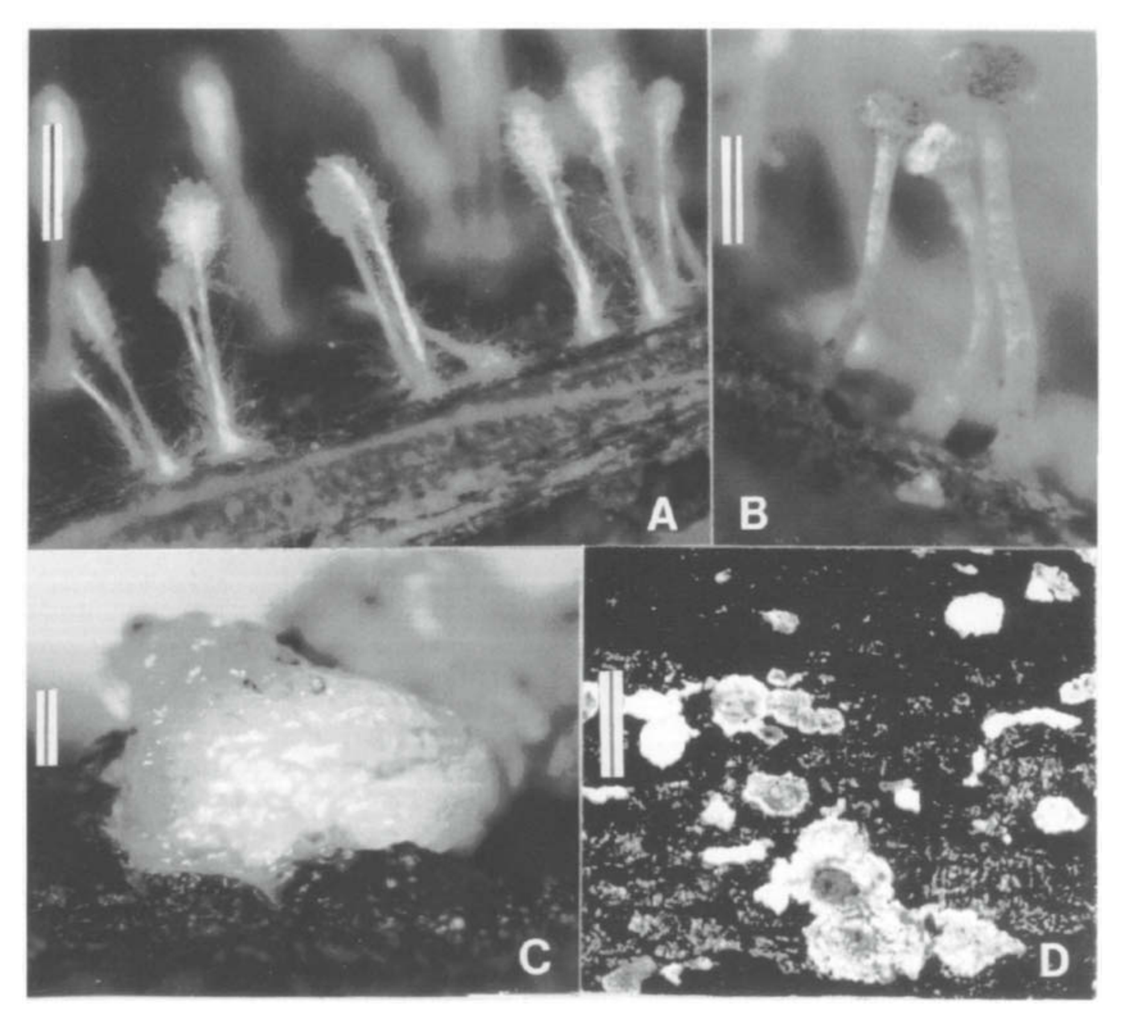


Fig. 292. (A) *Agaricostilbum hyphaenes* (Hariot & Patouillard) Oberwinkler & Bandoni (Agaricostilbales), basidiomata, RJB 7760, Thailand. Bar = 0.5 mm. (B) *Chionosphaera apobasidialis* Cox (Atractiellales), basidiomata, A-A and RJB 9691, Costa Rica. Bar = 0.6 mm. (C) *Mycogloea* sp. (Platygloeales), RJB 7984, British Columbia. Bar = 0.2 mm. (D) *Corticium*-like basidiomata of an undescribed *Septobasidium* sp. (Septobasidiales), A-A and RJB 11075, Thailand. Bar = 1.0 cm.

4.2.6. Atractiellales sensu lato and Agaricostilbales: The order Atractiellales includes a heterogeneous assortment of fungi. Most species produce minute synnema-like basidiomes (Fig. 292). An exception is *Atractogloea stillata* Oberwinkler & Bandoni, which has small pustulate basidiomes. *Atractiella* species described to date are characterized by strictly hyphal growth. The species of *Agaricostilbum,* placed in the Fungi Imperfecti prior to 1980 (Wright et al. 1981), are xerophytic species growing especially on attached or fallen dead palm parts, or, infrequently, on other monocotyledones. Basidia (Fig. 293) predominantly are produced at or near the apices of synnemata-like structures. The basidia do not arise from teliospores, are mostly four-celled, and the cells bud off multiple spores as in many smuts. Unlike smut basidia, however, the walls thicken with age and the budded basidiospores (also with walls that tend to thicken) mostly remain weakly attached in clusters on their basidial cells. The spores germinate to form rather slimy yeast colonies, but development beyond that is unknown. *Agaricostilbum* species are not closely related to other Atractiellales, and they have been segregated into a new order, Agaricostilbales (Oberwinkler and Bauer 1989). This classification is supported by the study of nuclear division (Bauer et al. 1992).

Chionosphaera apobasidialis, Stilbum vulgare and *Fibulostilbum phylacicola* (Chionosphaeraceae) are all presumed to be mycoparasites. *C. apobasidialis* is found with various dematiaceous fungi on bark of standing trees (Cox 1976). The species is sometimes present on branches falling from the jungle canopy in Costa Rica (R.J. Bandoni, unpublished observation). This species sporulates readily in culture in the absence of associated fungi (Oberwinkler and Bandoni 1982b), producing clavate nonseptate basidia (Fig. 294), each of which bears a terminal cluster of basidiospores. Germination is by budding. *Stilbum vulgare,* reported from a variety of substrates, is a rather variable species with 2-celled auricularioid basidia (Fig. 295). The basidiospores give rise to yeast colonies (Seifert et al. 1992). Mass spore

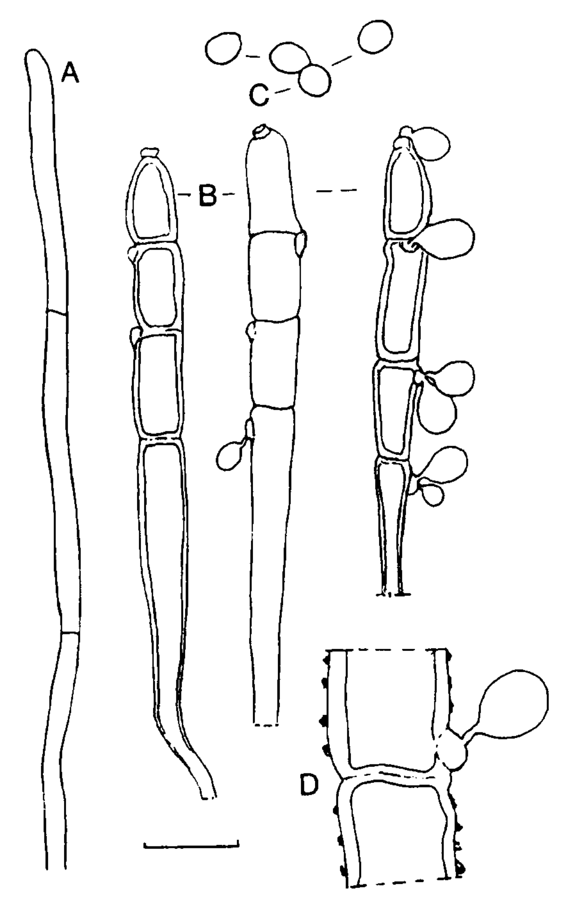


Fig. 293. *Agaricostilbum hyphaenes* (Hariot & Patouillard) Oberwinkler & Bandoni (Agaricostilbales). (A) Hyphidium. (B) Three mature basidia, two with attached basidiospores. (C) Basidiospores. (D) Diagrammatic portion of basidium showing thick rough walls, budding locus, and attached basidiospore, note very narrow attachment characteristic of many spores in this species. RJB 7760, Thailand. Bar = 10 μm.

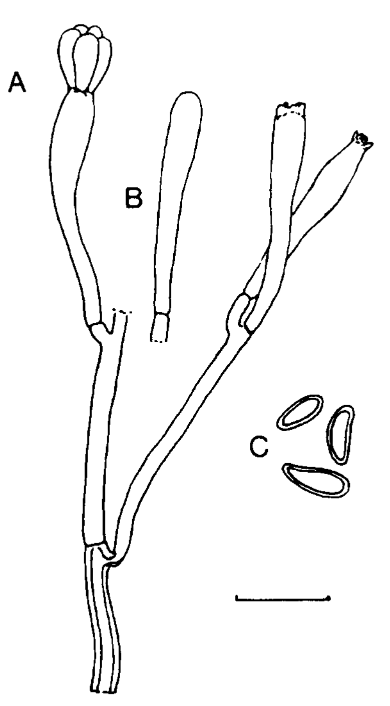


Fig. 294. *Chionospheara apobasidialis* Cox (Atractiellales). (A) Fertile hypha and three basidia, that on the left with attached spores. (B) Young basidium. (C) Basidiospores. A-A and RJB 9691, Costa Rica. Bar = 10 μm.

isolates frequently produce basidiomata in culture, but the details have not been studied. Unusual here is the near absence of a spreading dikaryotic mycelium, and the basidiomata arise at the margins of yeast colonies (R.J. Bandoni, unpublished observation). A strain of this species, isolated from basidiomes of *Yucca brevifolia,* produced narrow hyphae with haustoria at the surfaces of hyphae from a nearby *Cladosporium* sp. *Fibulostilbum phylacicola* produces basidiomata on and around ascomata of *Phylacia* spp. Attempts to culture the fungus have not been successful.

Atractogloea stillata apparently has been collected only once (Oberwinkler and Bandoni 1982c). Although budding was not noted in the original description, a yeast state was reported by Oberwinkler and Bauer (1989). It has a diglobular spindle pole body, and thus differs from other Atractiellales sensu lato. According to Prillinger et al. (1993), the cell wall carbohydrate pattern agrees with the pattern in *Cystofilobasidium* species and *Tremella mesenterica.*

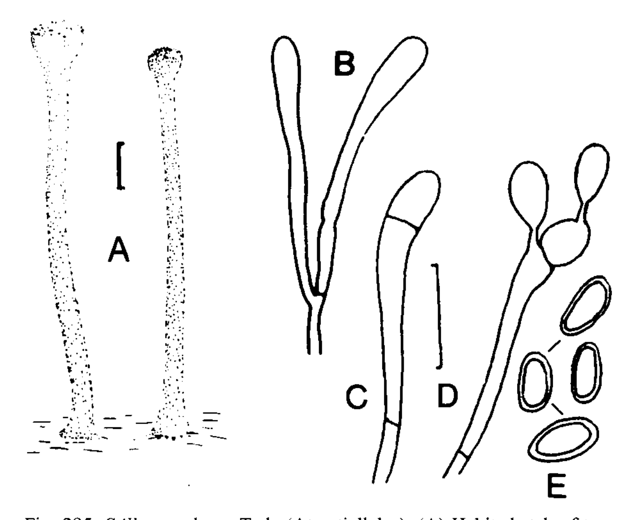


Fig. 295. *Stilbum vulgare* Tode (Atractiellales). (A) Habit sketch of two basidiomata. Bar = 0.1 mm. (B), (C) Developing basidia. Bar = 10 μm. (D) Mature basidium with attached basidiospores. (E) Basidiospores. J. Simpson and RJB 6852, Australia.

4.2.7. Septobasidiales: Basidiospores of *Septobasidium* species were first reported to bud by Couch (1938). All species are associated with scale insects on a variety of woody plants. An effused thallus develops on the bark or epidermal surface of the living plants; many scale insects dwell within the thallus or beneath it. Some individual scale insects are parasitized by *Septobasidium,*

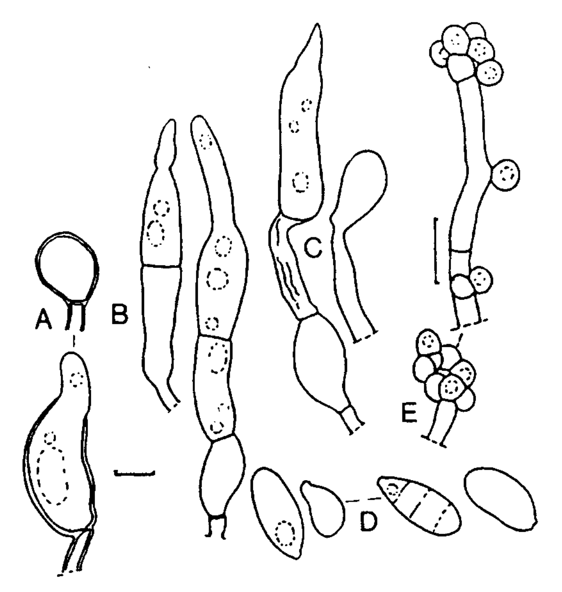


Fig. 296. *Septobasidium* sp. (A) Two probasidia. (B) Developing 2-celled basidia. (C) Mature basidium, one cell spent and collapsed, and portion of a sterigma with developing spore. (D) Four basidiospores, one of which has become septate during germination to produce yeast phase. (E) Hyphae from context bearing conidial clusters. A-A and RJB 11074, Thailand. Bars = 10 μm.

the fungus producing coiled haustoria within the insect gut. However, most of the insects are unparasitized, moving about freely and reproducing in or under the thallus. Similar haustoria are characteristic of several other auricularioid fungi. Details of the morphology, life histories and biology were included in the monograph by Couch (1938). Many species have typical transversely septate, four-celled basidia similar to those of the rusts, but one- or two-celled basidia (Fig. 296) are characteristic of some species. The basidiospores are born on sterigmata and are abstricted from basidia scattered at the basidiome surface, i.e., not forming a compact hymenium. At germination, yeast colonies develop in species of *Septobasidium* (Couch 1938) and in *Auriculoscypha anacardiicola* Reid & Manimohan (Lalitha and Leelavathy 1990). The cupulate basidiomata of *A. anacardiicola* arise from small tubercles, each of which contains a female coccid.

4.2.8. Platygloeales: The Platygloeales include a variety of fungi with mostly four-celled "auricularioid" basidia, mostly abstricted basidiospores, and effused, pustulate, or clavarioid basidiomes. The ordinal name was badly selected as there is considerable disagreement over the choice of *Platygloea* vs. *Achroomyces* for the species which provides the basionym (*Platygloea tiliae*).

The species are saprobes on wood or they parasitize fungi, ferns, mosses, or vascular plants. Most species have haploid hyphal phases, so far as is known, but *Cystobasidium lasioboli* that parasitizes *Lasiobolus* sp. on dung, was reported to have a yeast state (de Lagerheim 1898), as does *Platygloea fimetaria*[1] (Fig. 297) (Bandoni 1984). *Colacogloea (Platygloea) peniophorae* (Fig. 298), which parasitizes corticiaceous hosts, produces abundant dikaryotic conidia and auricularioid basidia on the host hymenium.

The yeast state in *Platygloea fimicola* is pink (R.J. Bandoni, unpublished observations), whereas that of *C. peniophorae* is white. Yeast states also occur in *Tjibodasia pezizoidea* Holtermann (Holtermann 1898) and in *Mycogloea* spp. (Olive 1950, Bandoni 1984).

Mycogloea includes four described species with gelatinous pustulate basidiomata bearing abundant basidia (Fig. 299). The basidia, cylindric and 2–4-celled, are deciduous at maturity and are thus important disseminules. Unusual is the ability of the basidia to produce either sessile, passively released basidiospores or ballistospores on sterigmata (Olive 1950).

4.2.9. Tremellales: The Tremellales include three families, the largest of which, the Tremellaceae, comprises 5 genera and ca. 500 binomials, of which perhaps 150 are distinct species. Primarily on the basis of field observations, the species are thought to be mycoparasites that occur on ascomycetes or basidiomycetes, especially those colonizing recently killed woody twigs, and on lichens. Species of *Tremella* occur in dacrymycetaceous basidiocarps (Figs. 300, 301), on hymenia of polypores,

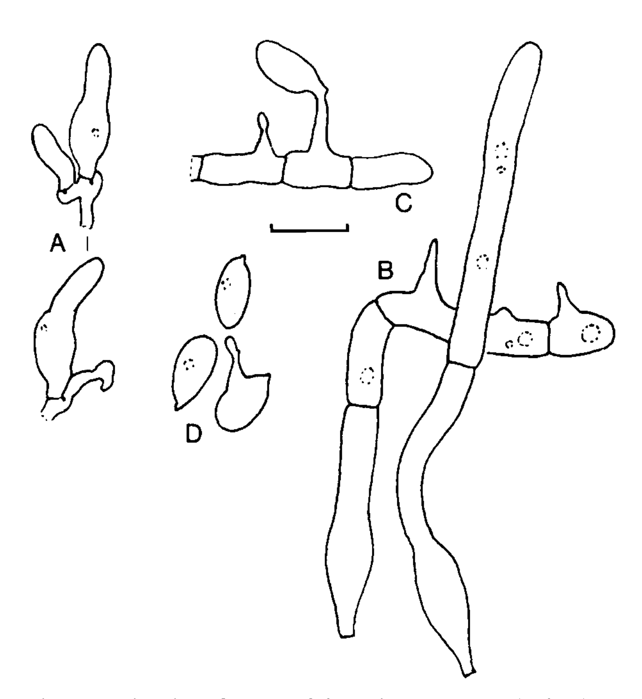


Fig. 297. *Platygloea fimetaria* (Schumacher:Persoon) Höhnel (Platygloeales). (A) Thin-walled probasidia with basidial initials. (B) Developing basidia. (C) Portion of mature basidium with attached basidiospore. (D) Basidiospores, one germinating by repetition. RJB 693, Canada. Bar = 10 μm.

[1] *Platygloea fimetaria* may be a synonym of *Cytobasidium lasioboli*, but this is not yet certain.

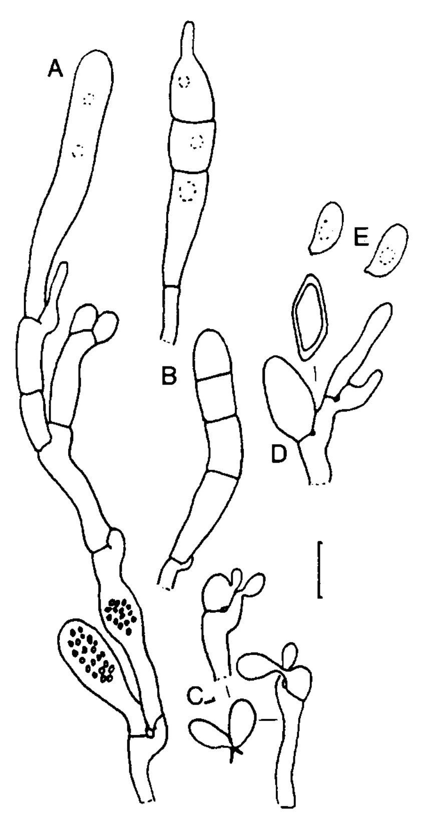


Fig. 298. *Colacogloea peniophorae* (Bourdot & Galzin) Oberwinkler & Bandoni. (A) Fertile hypha with basidium (top) and lateral branch with conidiogenous cells. (B) Mature 4-celled basidia, one with developing epibasidium. (C) Blastic conidial production and a pair of freed conidia. (D) Second form of conidial development and freed conidium. (E) Two basidiospores. RJB 74-7, Iowa. Bar = 10 µm.

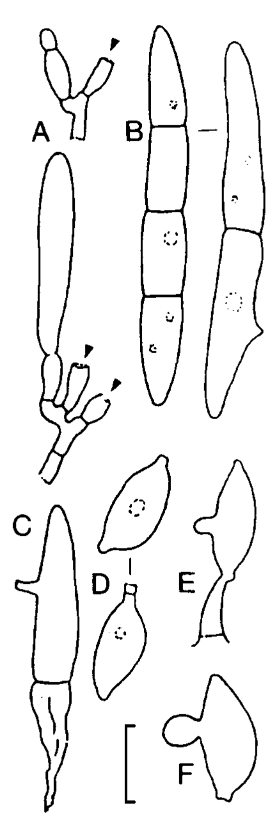


Fig. 299. A–B *Mycogloea* sp., RJB 7984, Canada. (C)–(F) *M. tahitiensis* Olive, RJB 7270, Japan. (A) Two fertile branches showing the minute probasidia and young developing basidia. Note the probasidial vestiges (arrows) left after basidia are released. (B) Two mature detached basidia. (C) Portion of mature basidium with one spent cell and the second with a sterigma. (D) Two basidiospores. (E) Basidiospore attached to sterigma and apparently germinating. (F) Budding basidiospore. Bar = 10 µm.

on hymenia of corticioid fungi (Fig. 302), growing from perithecial ostioles or on pyrenomycete stromata, in apothecia of bitunicate ascomycetes, and on lichen thalli. Some species (e.g., *Tremella mesenterica,* Fig. 302), grow on wood often with no conspicuous physical contact with other fungi. In *T. mesenterica,* however, haustorial branches can often be found attached to structures of nearby fungi. *Tremella* is not a natural genus as is apparent from both morphological studies and biochemical analyses. However, few species have been examined intensively by any method.

Bulleromyces Boekhout & A. Fonseca (Boekhout et al. 1991a), a recently added taxon in the Tremellaceae, is based upon a teleomorph obtained by mating strains of *Bullera alba.* The single species, *Bulleromyces albus,* produces dikaryotic hyphae when compatible strains are mated in culture. Basidiocarps are not produced, but typical tremellaceous basidia develop on the hyphae, which may produce buds or ballistospores. Other characteristics are the presence of *Tremella*-like haustorial branches, dolipores with a cupulate parenthesome, assimilation of *myo*-inositol and D-glucuronate, and the production of extracellular starch-like compounds. Partial 26S rRNA se-

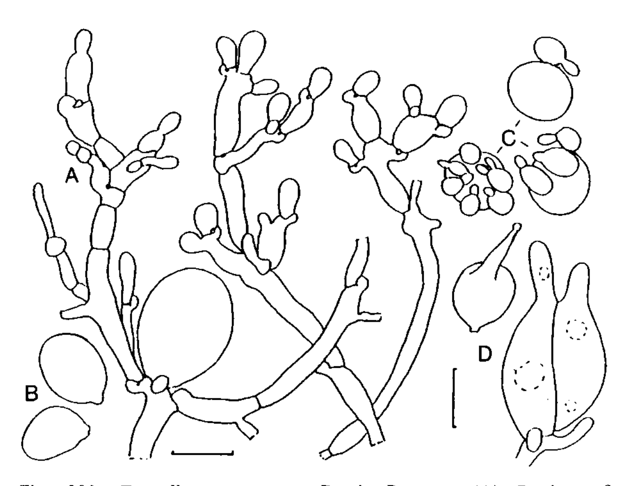


Fig. 300. *Tremella mesenterica* Retzius:Persoon. (A) Portion of hymenium showing a single probasidium and hyphae with conidiogenous cells. (B) Two basidiospores. (C) Germinating basidiospores, the retained primary outgrowths producing buds. (D) Germinating basidiospore and mature basidium. A–B, Bethel, San Diego, NYBG; C–D, RJB 7934 and 1839, respectively, Canada. Bars = 10 µm.

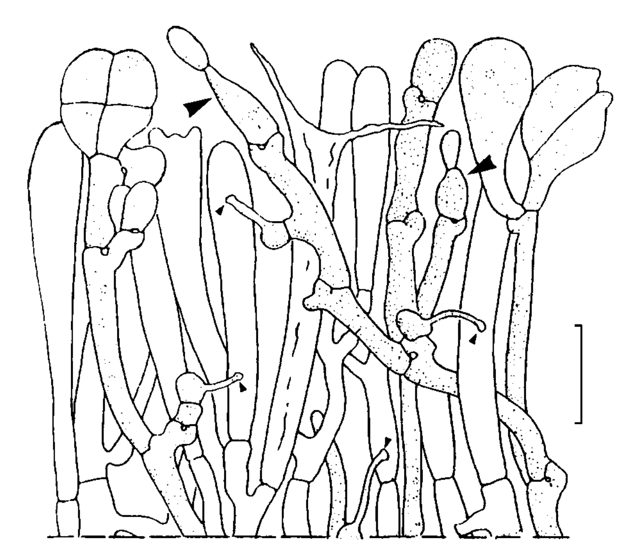


Fig. 301. *Tremella mycophaga* var. *obscura* Olive growing (stippled structures) in basidiome of *Dacrymyces stillatus* Nees:Fries. The hyphae bear basidia and phialide-like conidiogenous cells (upper arrow). Note haustorial branches and attachment of filaments to host basidia (lower arrows). L.S. Olive, Type, Georgia. Bar = 10 μm.

quences of the anamorph *Bullera alba*, suggest *B. albus* to have a close phylogenetic relationship with *Cryptococcus laurentii* (Fell et al. 1992). Van de Peer et al. (1992) found *Bullera alba* to be a sister species of *Filobasidiella neoformans* based on the entire 18S ribosomal RNA sequences.

Trimorphomyces, a monotypic genus, differs from *Tremella* by having a distinctive dikaryotic yeast state in addition to the haploid one (Figs. 303, 304). The minute basidiomes typically have mixed conidia and basidia, with the latter generally present in low numbers. Conidiogenesis is identical to bud development in the dikaryotic yeast cells. The conidia can give rise directly to a dikaryotic yeast state upon germination.

Phyllogloea tremelloidea Lowy, a species with "auricularioid" basidia, but with *Tremella*-type dolipore septa, belongs in the Tremellaceae (F. Oberwinkler, personal communication), as do the species of *Xenolachne* Rogers. The latter have haustorial branches of the *Tremella* type and are parasites of discomycetes. Yeast phases are unknown in these species, although budding has been observed in both groups.

Fig. 302. Tremellales. (A) *Sirobasidium magnum* Boedijn basidiocarp, A-A and RJB 9082, Taiwan. Bar = 1 mm. (B) Young basidiome of *Sirotrema pusila* Bandoni developing in the hymenium of a *Lophodermium ?pacificensis* ascoma, RJB 2233, Canada. Bar = 0.5 mm. The swelling basidiome destroys much of the ascocarp and often obscures the entire structure at maturity. (C) Basidiome of *Tremella mesenterica* Retzius:Persoon, RJB 7938. Bar = 1 cm. (D) Basidiomata of *Tremella mycetophilloides* Kobayasi (arrows) growing on hymenia of young *Aleurodiscus amorphus* (Persoon:Fries) Schröeter, basidiocarps, RJB 5277, Canada. Bar = 0.5 mm.

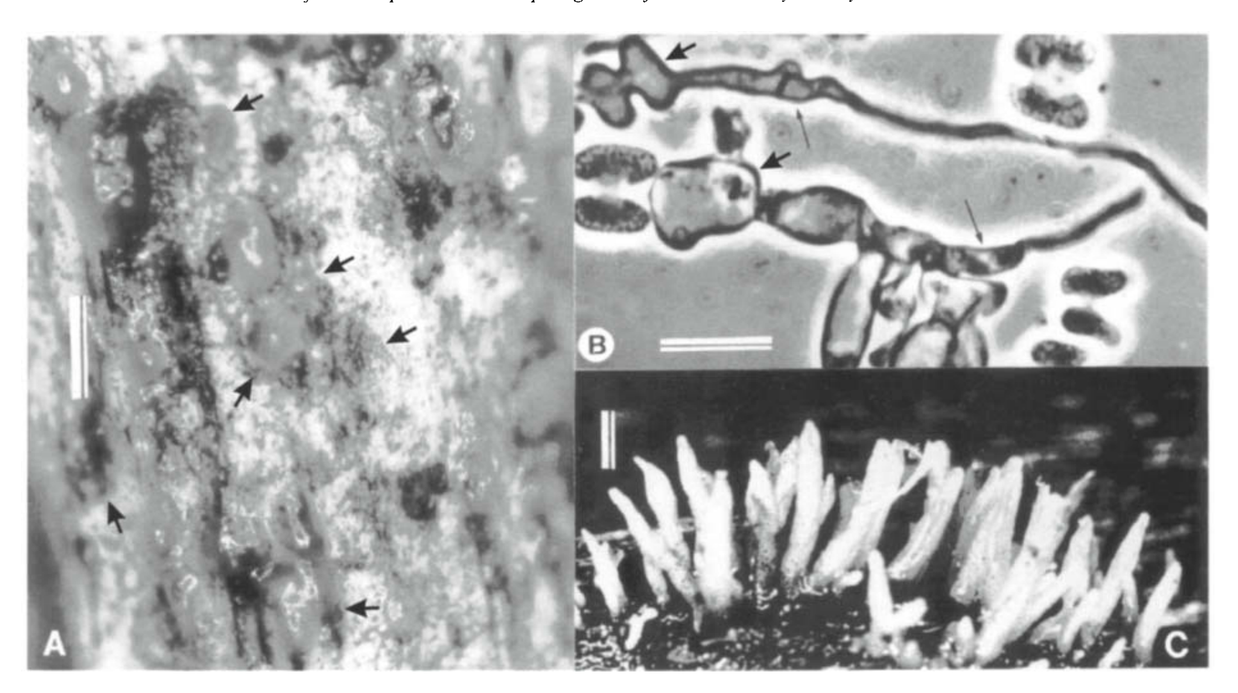


Fig. 303. *Trimorphomyces papilionaceus* Bandoni & Oberwinkler. (A) Gelatinous basidiomata scattered on a small culm of a *Sasa* sp. (some marked by arrows), RJB 7526, Japan. Bar = 0.5 mm. (B) Two dikaryotic yeast cells (arrows) germinating in the presence of host hyphae (not visible); the lower cell is much distorted, its form characteristic of older cells that have produced many buds (a mature bud is attached to it on the left). Each germinating cell has produced a short hypha and haustorial branch; ungerminated dikaryotic yeast cells are present on the right, culture of RJB 6646, Type, Canada. Bar = 5 µm. (C) *Holtermannia* sp., basidiocarps, A-A and RJB 9832, Thailand. Bar = 0.5 mm. Photo courtesy of Dr. T. Flegel.

Holtermannia species have basidiomes composed of aggregates of erect, simple or branched teeth. Although mycoparasitism was not mentioned by Kobayasi (1937) in his monograph, a common Thai species occurs only on shelving polypore basidiocarps or on wood heavily decayed by a white pocket rot fungus (R.J. Bandoni, unpublished observations).

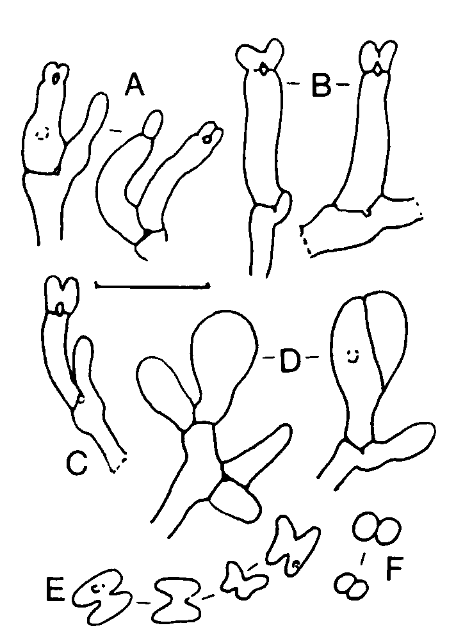


Fig. 304. *Trimorphomyces papilionaceus* Bandoni & Oberwinkler. (A)–(C) Conidiogenous cells and developing dikaryotic conidia from basidiome. (D) Two basidia. (E) Face view of H-shaped conidia. (F) Conidia in end view, RJB 7526, Japan. Bar = 10 µm.

Species of *Sirotrema* Bandoni (1986) grow in the hymenia of phacidiaceous apothecia (Figs. 302, 305), producing minute gelatinous basidiomata. The basidia often occur in short chains and the epibasidia are retained on the basidia. *Sirotrema* has some intermediate characteristics between the Tremellaceae and Sirobasidiaceae which, taken with other intermediate taxa currently placed in *Tremella,* suggests that the family Sirobasidiaceae is superfluous. However, recent molecular studies (E.C. Swann, personal communication) indicate a more distant relationship and additional analyses are needed.

The second family of Tremellales with a yeast state, the Sirobasidiaceae, currently contains only two genera, *Sirobasidium* and *Fibulobasidium.* In *Sirobasidium,* basidia are catenate (Figs. 306, 307), maturing basipetally from successive lower cells of fertile hyphae. The basidiospores, clavate to fusiform and passively released, are probably homologs of *Tremella* epibasidia (Bandoni 1957). *Fibulobasidium* basidia develop by direct expansion of successively formed clamp connections with the basidia laterally fused (Fig. 308). Basidiomata of *Sirobasidium* spp. are exposed and conspicuous, whereas those of *Fibulobasidium* species are subcortical on recently killed branches. As in *Sirobasidium* , the fusiform, passively released basidiospores of *F. inconspicuum* can germinate either by budding or by producing a sterigma and ballistospore.

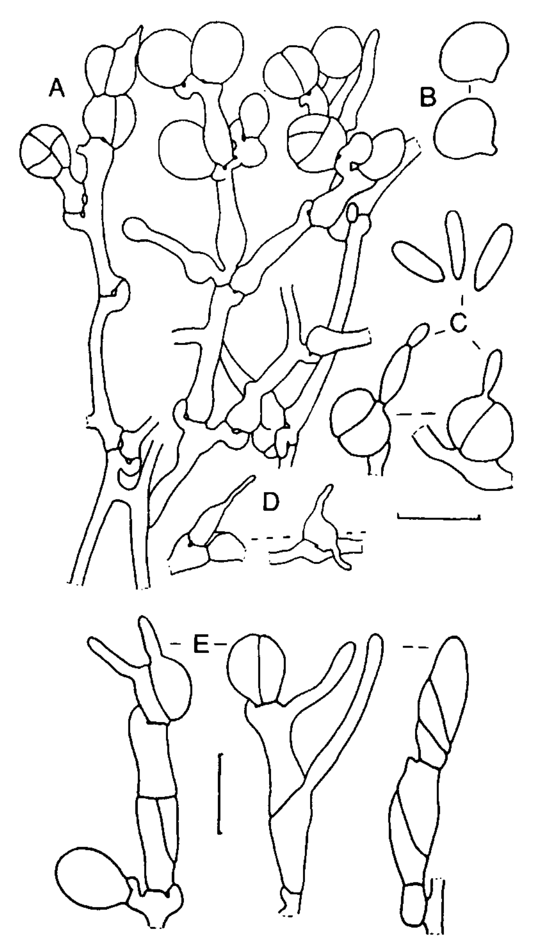


Fig. 305. (A)–(D) *Sirotrema parvula* Bandoni. (A) Fertile hyphae with basidia and probasidia (note catenate basidial pair, upper left). (B) Typical (abstricted) basidiospores. (C) Production of passively-released basidiospores. (D) Haustorial branches. (E) Basidia of *S. pusilla* Bandoni in catenate pairs, the intercalary ones functional. A–D, RJB 7478; E, RJB 2233, Canada. Bars = 10 µm.

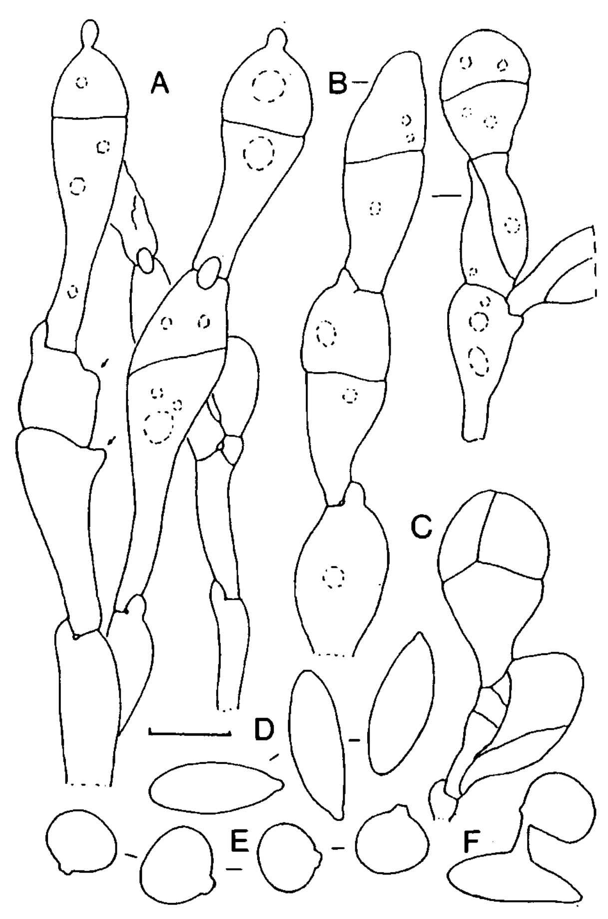


Fig. 307. *Sirobasidium brefeldianum* Möller. (A), (B) Chains of two-celled basidia (typical of this species). (C) Three-(? or four-)celled basidia (atypical). (D) Basidiospores. (E) Ballistospores. (F) Developing ballistospore. A–B + D–F, A-A and RJB 9708; C, A-A and RJB 9495A, Costa Rica. Bar = 10 µm.

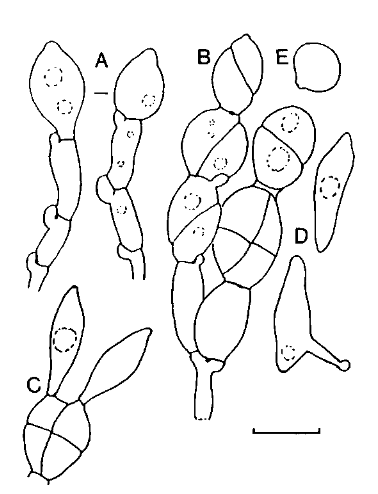


Fig. 306. *Sirobasidium magnum* Boedijn. (A) Young basidial chains, the terminal cells partially expanded. (B) Mature basidial chains. (C) Basidium with attached basidiospores. (D) Basidiospores, the lowermost germinating by ballistospore formation. (E) Ballistospore. A-A and RJB 9082, Taiwan. Bar = 10 µm.

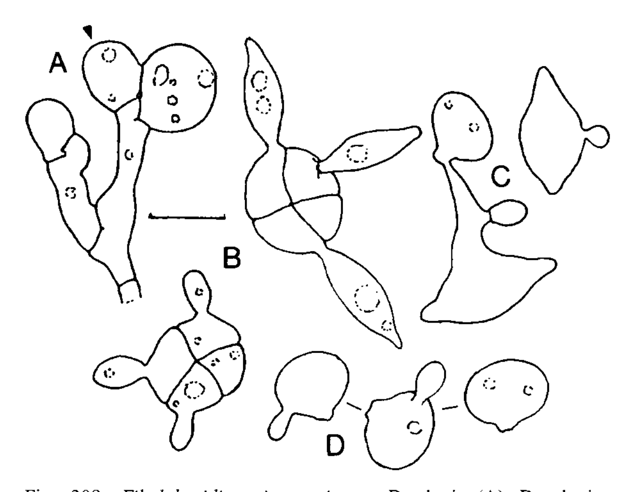


Fig. 308. *Fibulobasidium inconspicuum* Bandoni. (A) Developing basidia, the youngest (arrow) in the pair formed by direct expansion of the basal clamp of the first basidium. (B) Two detached basidia with developing basidiospores. (C) Two germinating basidiospores, that on the left germinating by both budding and ballistospore formation. (D) Ballistospores, two germinating by budding. RJB 6075, Louisiana. Bar = 10 µm.

Itersonilia Derx, an anamorphic genus placed by von Arx et al. (1977) in the Filobasidiaceae, appears to be related to the Tremellales/Filobasidiales because of its dolipore septum and the presence of xylose in whole-cell hydrolyzates. However, a parenthesome is absent, and molecular data suggest a more distant relationship (Fell et al. 1995). *Sterigmatosporidium polymorphum* Kraepelin and Schulze (1982) also seems related to the Tremellales or Filobasidiales.

4.2.10. Filobasidiales: The Filobasidiales, which appear to be closely related to the Tremellales, lack conspicuous basidiocarps and have holobasidia and passively released basidiospores. They have the *Tremella*-type haustorial branches. Most species with yeast states are placed in the family Filobasidiaceae L.S. Olive (1968) but some are also classified in the Syzygosporaceae Jülich. The order is restricted to teleomorphic, heterobasidiomycetous yeasts and yeastlike fungi. Budding is enteroblastic and hyphae usually form clamp connections and haustorial branches; teliospores are usually absent; holobasidia are clavate with sessile basidiospores; cell walls are lamellate and hyphal septa, if perforate, have dolipores; parenthesomes, if present, are cupulate; xylose is present in whole-cell hydrolyzates. The type genus is *Filobasidium*; other genera belonging here are *Filobasidiella* and probably *Cystofilobasidium, Mrakia* and *Xanthophyllomyces* Golubev (Golubev 1995).

Based on basidial morphology and the manner in which dikaryotization occurs in *Filobasidium floriforme,* Olive (1968) placed the Filobasidiaceae in the Ustilaginales, which in his concept included both Ustilaginaceae and Tilletiaceae. The family was established to accommodate *Filobasidium floriforme,* found in a culture dish in which dead florets of *Erianthus giganteus* had been placed (Olive 1968). Three species of *Filobasidium* on *Yucca brevifolia* (Fig. 309) produce aggregates of basidia that can readily be seen with low magnification (Bandoni et al. 1991). *Filobasidiella,* containing the only animal parasitic species known in the group, has basidia like those in *Filobasidium* spp., but basidiospores develop in basipetal chains. Haustorial branches are found in *Filobasidiella neoformans,* suggesting that the species primarily is a fungal parasite and secondarily acts as an opportunistic animal pathogen.

Thick-walled teliospores are present in *Cystofilobasidium,* but are absent in the remaining genera of the Filobasidiales. *Cystofilobasidium* has Co-Q 8, *Filobasidiella* and *Xanthophyllomyces* Co-Q 10 (Sugiyama et al. 1985), and *Filobasidium* either Co-Q 9 or 10 (Yamada and Kondo 1972a, 1973).

Filobasidium capsuligenum and *Xanthophyllomyces dendrorhous* (anamorph *Phaffia rhodozyma*) are able to ferment glucose, whereas the remaining taxa are unable to ferment carbohydrates. All taxa assimilate *myo*-inositol

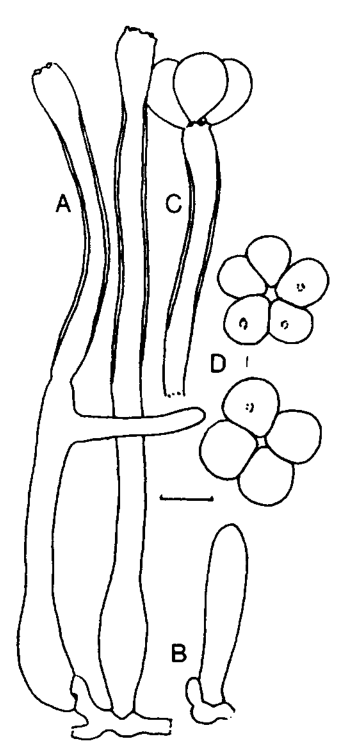


Fig. 309. *Filobasidium globosporum* Bandoni & Oberwinkler (Filobasidiales). (A) Two spent basidia. (B) Young basidium. (C) Upper portion of basidium with spores still attached. (D) Adherent basidiospores as released from basidia. A-A and RJB 8197B, California. Bar = 10 μm.

and D-glucuronate, and produce extracellular starch-like compounds.

The species are either homothallic or heterothallic, the latter apparently controlled by biallelic unifactorial mating systems. Upon conjugation, clamped dikaryotic hyphae develop, which may form haustorial branches. Basidia are clavate, and basidiospores are formed terminally. Species of the Filobasidiaceae differ from the Sporidiobolaceae by the presence of dolipores and the presence of xylose in their cell walls (Sugiyama et al. 1985, Boekhout et al. 1992b, 1993, Prillinger et al. 1993).

Cox (1976) proposed transferring the Filobasidiaceae to the Aphyllophorales (Holobasidiomycetes/Homobasidiomycetes), because of the presence of holobasidia. Jülich (1981) established the order Filobasidiales to accommodate the Filobasidiaceae, without indicating the demarcation between Filobasidiales and Tremellales. The Filobasidiaceae were considered by Oberwinkler (1987) to be sufficiently close to the Tremellales to constitute a family in that order. This interpretation has since been supported by comparison of 18S rRNA gene sequencing studies (Swann and Taylor 1993). Subdivision of the basidiomycetes based on basidial morphology is no longer accepted because basidial form and septation appear to be less reliable criteria than septal pore ultrastructure and ribosomal DNA sequences for determining relationships among these fungi (Bandoni 1984, Walker 1984, Gottschalk and Blanz 1985, Fell et al. 1992). The distinction of Filobasidiales and Tremellales is not

Table 66
Summary of basidiomycetous yeast groups

Characteristic	Order	Family	Genus
Teleomorphic taxa			
I. With "simple" septal pores			
A. Basidia cylindric, transversely septate			
	Ustilaginales		
		Ustilaginaceae	
			Microbotryum
			Schizonella
			Sorosporium
			Sphacelotheca
			Sporisorium
			Ustilago
			Ustilentyloma and probably with *Ustilago*-type basidia[a]
	Sporidiales		
		Sporidiobolaceae	
			Leucosporidium
			Rhodosporidium
			Sporidiobolus
			?*Erythrobasidium*
			?*Kondoa*
			?*Sakaguchia*
	Platygloeales		
		Cystobasidiaceae	
			Colacogloea
			Cystobasidium
			Kriegeria[b]
			Mycogloea
			Occultifer
			Tijbodasia
	Septobasidiales		
		Septobasidiaceae	
			Auriculoscypha
			Coccidiodictyon[c]
			Ordonia[c]
			Septobasidium
	Atractiellales[d]		
		Chionosphaeraceae	
			Chionosphaera
			Stilbum
		Atractogloeaceae	
			Atractogloea
	Agaricostilbales		
		Agaricostilbaceae	
			Agaricostilbum
B. Basidia globose, nonseptate			
	Graphiolales		
		Graphiolaceae	
			Graphiola
C. Basidia cylindric, nonseptate			
	Cryptobasidiales		
		Cryptobasidiaceae	
			Conyodyctum
			Cryptobasidium
		Microstromaceae	
			Microstroma
	Exobasidiales		
		Exobasidiaceae	
			Brachybasidium
			Dicellomyces
			Exobasidiellum
			Exobasidium
			Laurobasidium
II. With dolipore septa, parenthesomes cupulate			
A. Basidia "cruciate-septate"			
	Tremellales		
		Sirobasidiaceae	
			Fibulobasidium
			Sirobasidium
		Tremellaceae	
			Bulleromyces
			Itersonilia[e]
			Holtermannia
			Phyllogloea
			Sirotrema
			Tremella
			Trimorphomyces
B. Basidia aseptate			
	Filobasidiales		
		Filobasidiaceae	
			Cystofilobasidium[f]
			Filobasidiella
			Filobasidium
			Mrakia
			Xanthophyllomyces
		Syzygosporaceae	
			Christiansenia
			Syzygospora
Anamorphic taxa			
		Sporobolomycetaceae	
			Bensingtonia pro parte
			Kurtzmanomyces
			Rhodotorula pro parte
			Sporobolomyces pro parte
			Sterigmatomyces
		Cryptococcaceae	
			Bullera
			Cryptococcus
			Fellomyces
			Kockovaella
			Phaffia
			Trichosporon
			Tsuchiyaea
			Udeniomyces
			?*Hyalodendron*
			?*Moniliella*

continued on next page

Table 66, notes

[a] Although economically important smuts have been studied in detail, type of teliospore germination is unknown in many species. Direct conjugation of basidial cells occurs in some taxa with *Ustilago*-like basidia and this can result in the complete absence or infrequent occurrence of a yeast state.

[b] *Kriegeria (Xenogloea) eriophori* (monotypic) parasitizes monocots; its relationship to the mycoparasitic taxa placed in the Cystobasidiaceae and to most other Platygloeales, may be distant.

[c] Yeast states probably occur in these two genera.

[d] Basidia can be cylindric, transversely septate (e.g., in *Stilbum*), or clavate, holobasidia (e.g., as in *Chionosphaera*).

[e] *Itersonilia perplexans* appears to belong in this group (but see also under f), but basidia have not been found. Because of the known features, and the rather isolated position among anamorphic yeast groups, it is classified with the Tremellales here.

[f] *Cystofilobasidium* has thick-walled teliospores which germinate with holobasidia. Because of biochemical traits such as cell wall composition, dolipores without parenthesomes, and apparently unique molecular characteristics, we tentatively place the genus here. Recent partial 26S rDNA sequences (Fell et al. 1992, 1995) suggest a more distant relationship between *Cystofilobasidium, Mrakia, Xanthophyllomyces* and *Itersonilia* on one side with the Tremellales and the genera *Filobasidium* and *Filobasidiella* on the other.

supported by partial 26S rRNA sequences (Fell et al. 1992, 1995, Guého et al. 1993), nor by 18S rDNA sequences (Swann and Taylor 1993). For definitive conclusions, more taxa need to be studied with molecular methods. Therefore, and for reasons of convenience, we maintain the Filobasidiaceae in the Filobasidiales, as they are represented by a number of morphologically uniform genera, which share holobasidia, lack ballistospores, mostly lack basidiocarps and frequently have a different ecology. Recent molecular analyses suggest that the teliospore-forming taxa, such as *Cystofilobasidium* and *Mrakia,* do not form a monophyletic group with *Filobasidiella* and *Filobasidium* (Fell et al. 1995, Swann and Taylor 1993, 1995a).

Filobasidium and *Filobasidiella* are characterized by haustorial branches (Olive 1968, Kwon-Chung 1975) similar to those seen in *Tremella,* and the septal pore apparatus is also similar in the two groups (Moore and Kreger-van Rij 1972, Bandoni 1987, Moore 1978, 1987b, Khan 1976, Patrignani et al. 1984). Moreover, species of *Filobasidium* and *Filobasidiella* produce an extracellular polysaccharide with a chemical structure similar to that occurring in *Tremella* species (Battacharjee et al. 1978), and extracellular enzymes, such as deoxyribonuclease produced by *Filobasidium* and *Filobasidiella* are also found in *Tremella* species. The main differentiating character between the Filobasidiales and Tremellales is the presence of holobasidia in the first group, whereas the second group usually has cruciately septate basidia (Oberwinkler 1987, Bandoni 1987).

Syzygospora and *Christiansenia* species parasitize basidiomycetes or ascomycetes, inducing tumor-like swellings in some hosts, e.g., *Christiansenia* spp. on *Collybia dryophila* (Ginns and Sunhede 1978). Basidiocarps and yeast states of *Syzygospora alba* Martin are similar to those of some *Tremella* spp. Haploid states of *Christiansenia* species examined by Ginns (1986) were "yeastlike" and similar to those in some species of *Exobasidium,* but a yeast state is known in *C. pallida* (Oberwinkler et al. 1984). The haustorial apparatus in this species is similar to that in tremellaceous fungi (Bauer and Oberwinkler 1990b).

In the previous edition of this book, the genus *Chionosphaera* was included in the family Filobasidiaceae based on Cox's description of the genus. *Chionosphaera,* however, has been transferred to the Chionosphaeriaceae (Atractiellales, see above) by Oberwinkler and Bandoni (1982c). *Chionosphaera apobasidialis,* the type species, does not fit into the Filobasidiaceae, because of the presence of capitate basidiocarps, the absence of dolipores, and the inability to assimilate *myo*-inositol.

The monotypic genus *Rogersomyces* was placed in the family Filobasidiaceae by Crane and Schoknecht (1978). This genus does not fit into the Filobasidiaceae, since *R. okefenokeensis* produces holobasidia with basidiospores formed on sterigmata.

The system of classification accepted for heterobasidiomycetous taxa in the present edition of *The Yeasts,* is outlined in Table 66.

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Chapter 75

Diagnostic descriptions and key to presently accepted heterobasidiomycetous genera

T. Boekhout

Contents

1. Diagnostic descriptions

1.1. Teleomorphic genera

1.1.1. *Agaricostilbum*:

Vegetative reproduction: Yeast colonies are yellowish-cream and butyrous. Yeast cells are ellipsoidal-fusoidal, sometimes adhering in chains and with polar budding.

Sexual reproduction: Basidiocarps are synnemata-like. Hyphae lack clamp connections. Basidia are cylindrical, 4-celled and transversely septate, with the basidiospores laterally budding off (Figs. 292A, 293). Septal pores are "simple".

Physiology: Fermentation is absent. D-Glucuronate is assimilated, *myo*-inositol and nitrate are not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q is not determined. Xylose is absent in whole-cell hydrolyzates.

1.1.2. *Bulleromyces*:

Vegetative reproduction: Yeast colonies are cream-colored and butyrous to mucoid. Yeast cells are ellipsoidal, subglobose to cylindrical with polar budding; pseudohyphae may be present. Ballistoconidia are rotationally symmetrical.

Sexual reproduction: Basidiocarps are absent. Hyphae have clamp connections. Basidia are tremelloid with 2–4 cells. Septal pores are dolipores with cupulate parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated; nitrate is not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

1.1.3. *Chionosphaera*:

Vegetative reproduction: Yeast colonies are cream-colored and butyrous to mucoid. Yeast cells are ellipsoidal to allantoidal and have polar budding.

Sexual reproduction: Basidiocarps are stilbelloid, waxy-arid or subgelatinous. Hyphae lack clamp connections. Basidia are 1-celled and clavate, with sessile basidiospores (Figs. 292B, 294). Septal pores are "simple".

Physiology: Fermentation is absent. D-Glucuronate, *myo*-inositol and nitrate are not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Cell-wall composition is not determined.

1.1.4. *Cystofilobasidium*:

Vegetative reproduction: Yeast colonies are orange, salmon, cinnamon or pale. Yeast cells are ovoidal to elongate, and pseudohyphae may be present.

Sexual reproduction: Basidiocarps are absent. Hyphae may or may not have clamp connections. Endospores are sometimes present. Teliospores are present. Basidia are 1-celled, and clavate or capitate. Septal pores are dolipores without parenthesomes.

Physiology: Fermentation is usually absent. D-Glucuronate, *myo*-inositol and nitrate are assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-8 is formed. Xylose is present in whole-cell hydrolyzates.

1.1.5. *Erythrobasidium*:

Vegetative reproduction: Yeast colonies are orange-red. Yeast cells are ovoidal to elongate, and have multilateral budding.

Sexual reproduction: Basidiocarps are absent. Hyphae have pseudoclamp connections. Basidia are 1-celled. Septal pores are "simple".

Physiology: Fermentation is absent. D-Glucuronate and nitrate are assimilated, but *myo*-inositol is not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q- 10(H_2) is formed. Xylose is absent in whole-cell hydrolyzates.

1.1.6. *Fibulobasidium*:

Vegetative reproduction: Yeast colonies are grayish to brownish-yellow, and butyrous. Yeast cells are subglobose to globose. Ballistoconidia are rotationally symmetrical.

Sexual reproduction: Basidiocarps are pulvinate and gelatinous. Hyphae have clamp connections. Basidia are tremelloid, occur in chains and develop from successively formed basal clamp connections of earlier basidia (Fig. 308). Septal pore structure is not known.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated; nitrate assimilation is absent or weak. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 is formed. Cell-wall composition is not determined.

1.1.7. *Filobasidiella*:

Vegetative reproduction: Yeast colonies are whitish to yellowish-cream and usually mucoid. Yeast cells are globose, ovoidal, apiculate, and have polar or multilateral budding.

Sexual reproduction: Basidiocarps are absent. Hyphal colonies, if present, are whitish and velutinous. Hyphae usually have clamp connections. Basidia are 1-celled and stipitate-capitate with basipetally formed chains of basidiospores. Septal pores are dolipores with or without cupulate parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated; nitrate is not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

1.1.8. *Filobasidium*:

Vegetative reproduction: Yeast colonies are white, cream or pinkish and mucoid. Yeast cells are globose, ovoidal, ellipsoidal or elongate. Pseudohyphae may be present.

Sexual reproduction: Basidiocarps are absent. Hyphae have clamp connections. Basidia are 1-celled and clavate, with sessile basidiospores (Fig. 309). Septal pores are dolipores with or without cupulate parenthesomes.

Physiology: Fermentation may be present. D-Glucuronate, *myo*-inositol and nitrate are sometimes assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 or 10 are formed. Xylose is present in whole-cell hydrolyzates.

1.1.9. *Holtermannia*:

Vegetative reproduction: Yeast colonies are yellowish-white and mucoid. Yeast cells are ellipsoidal and have polar budding.

Sexual reproduction: Basidiocarps are erect, coralloid, gelatinous, and occur in dense aggregates (Fig. 303C). Hyphae have clamp connections. Basidia are tremelloid. Septal pore structure is not known.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated; nitrate is not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q is not determined. Xylose is present in whole-cell hydrolyzates.

1.1.10. *Leucosporidium*:

Vegetative reproduction: Yeast colonies are cream-colored and mucoid. Yeast cells are ovoidal to elongate and pseudohyphae may be present.

Sexual reproduction: Basidiocarps are absent. Hyphae usually have clamp connections. Teliospores are present. Basidia are transversely septate. Septal pores are "simple".

Physiology: Fermentation is absent. D-Glucuronate is sometimes assimilated; *myo*-inositol is not assimilated; nitrate is assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 or 10 are formed. Xylose is absent in whole-cell hydrolyzates.

1.1.11. *Mrakia*:

Vegetative reproduction: Yeast colonies are whitish or cream-colored and mucoid. Yeast cells are ovoidal to elongate, and pseudohyphae may be present.

Sexual reproduction: Basidiocarps are absent. Hyphae lack clamp connections. Teliospores are present. Basidia are 1-celled or transversely septate. Septal pores are dolipores without parenthesomes.

Physiology: Glucose and sometimes galactose may be fermented. D-Glucuronate and nitrate are assimilated, and *myo*-inositol is sometimes assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-8 is formed. Xylose is present in whole-cell hydrolyzates. Maximum temperature of growth is below 20°C.

1.1.12. *Rhodosporidium* (this treatment includes *Kondoa* and *Sakaguchia*):

Vegetative reproduction: Yeast colonies are orange, red or yellowish, and butyrous to mucoid. Yeast cells are ovoidal to elongate, and pseudohyphae may be present.

Sexual reproduction: Basidiocarps are absent. Hyphae may or may not have clamp connections. Teliospores are present. Basidia are transversely septate (Fig. 291). Septal pores are "simple".

Physiology: Fermentation is absent. D-Glucuronate and nitrate are sometimes assimilated, but *myo*-inositol is not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 or 10 are formed. Xylose is absent in whole-cell hydrolyzates.

1.1.13. *Sirobasidium*:

Vegetative reproduction: Yeast colonies are yellowish to yellowish-cream and mucoid. Yeast cells are ellipsoidal to subglobose and have polar budding. Ballistoconidia are bilaterally symmetrical. Pseudohyphae may be present.

Sexual reproduction: Basidiocarps are pulvinate to irregularly lobed or foliaceous. Hyphae have clamp connections. Basidia are tremelloid, occur in chains, and mature basipetally from contiguous cells of fertile hyphae (Figs. 302A, 306, 307). Septal pores are dolipores with cupulate parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated; nitrate is not assimilated. Starch-like compounds are produced. Diazonium blue B

and urease reactions are positive. Coenzyme Q and cell-wall composition are not determined.

1.1.14. *Sporidiobolus*:

Vegetative reproduction: Yeast colonies are reddish to pink. Yeast cells are ellipsoidal to elongate and have polar budding. Ballistoconidia are bilaterally symmetrical. Pseudohyphae may be present.

Sexual reproductions: Basidiocarps are absent. Hyphae usually have clamp connections. Teliospores are present. Basidia are usually transversely septate, but sometimes 1-celled. Septal pores are "simple".

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are not assimilated; nitrate is sometimes assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is lacking in whole-cell hydrolyzates.

1.1.15. *Sterigmatosporidium*:

Vegetative reproduction: Yeast colonies are cream-colored and butyrous. Yeast cells are ellipsoidal to elongate, and have polar budding, usually on stalks with the septa at the distal end. Pseudohyphae are present.

Sexual reproduction: Basidiocarps are absent. Hyphae have clamp connections. Dikaryotic chlamydospore-like structures are present. Basidia-like structures are 1-celled. Septal pore structure is not known.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated; nitrate is not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

1.1.16. *Tilletiaria*:

Vegetative reproduction: Colonies are grayish-brown and tough. Yeast cells are absent. Hyphae lack clamp connections. Chlamydospores are present. Ballistoconidia are bilaterally symmetrical.

Sexual reproduction: Basidiocarps are absent. Teliospores are echinulate and brown. Basidia are transversely septate. Septal pores are "primitive" dolipores without parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate is assimilated, but *myo*-inositol and nitrate are not assimilated. Starch-like compounds are not produced. Diazonium blue B reaction is not determined, but the urease reaction is positive. Coenzyme Q-10 is formed. Xylose is absent from whole-cell hydrolyzates.

1.1.17. *Tremella*:

Vegetative reproduction: Yeast colonies are cream-colored to pale brown and mucoid. Yeast cells are ellipsoidal, subglobose to globose, and have polar or multipolar budding. Pseudohyphae may be present.

Sexual reproduction: Basidiocarps are usually foliaceous, effuse, pulvinate or cerebriform, but may be absent in some taxa. Hyphae usually have clamp connections. Basidia are tremelloid (Figs. 290A–G, 300, 301, 302C,D). Septal pores are dolipores with cupulate parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol may or may not be assimilated, but nitrate is not assimilated. Starch-like compounds may or may not be produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

1.1.18. *Trimorphomyces*:

Vegetative reproduction: Haploid yeast colonies are pale pinkish-yellow to pinkish-brown and butyrous. The cells are subglobose, ellipsoidal, somewhat triangular, or elongate, and have polar or multipolar budding. Dikaryotic yeast colonies are of similar appearance, but the yeast cells here are H-shaped in most strains. The H-shaped cells develop synchronously as closely situated, mostly polar "twins"; they conjugate before maturity and are released as new H-shaped cells (Fig. 304).

Sexual reproduction: Basidiocarps are small, pulvinate, and occur associated with *Arthrinium* on bamboo and other grasses. Hyphae have clamp connections. Basidia are tremelloid (Figs. 303A,B, 304). Septal pores are dolipores with cupulate parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate is assimilated, *myo*-inositol is sometimes assimilated, but nitrate is not assimilated. Starch-like compounds are sometimes produced. Diazonium blue B and urease reactions are positive. Coenzyme Q is not determined. Xylose is present in whole-cell hydrolyzates.

1.1.19. *Xanthophyllomyces*:

Vegetative reproduction: Yeast colonies are orange to salmon-red and butyrous. Yeast cells are ellipsoidal. Pseudohyphae and chlamydospores may be present.

Sexual reproduction: Basidiocarps are absent. Hyphae are absent. Basidia originate from conjugation between a cell and its bud, and are 1-celled.

Physiology: Fermentation is present. D-Glucuronate is assimilated, but *myo*-inositol and nitrate are not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

1.2. Anamorphic genera

1.2.1. *Bensingtonia*:

Vegetative reproduction: Colonies are whitish, pinkish, ochraceous, brown to grayish-red, and butyrous. Yeast cells are ellipsoidal, ovoidal to elongate, and usually have polar budding. Ballistoconidia are bilaterally symmetrical. Chlamydospores, hyphae and pseudohyphae may be present. Septal pores, if present, are "simple" (teliospore formation has been observed in matings of *B. intermedia,* personal communication, W.I. Golubev).

Physiology: Fermentation is absent. D-Glucuronate and

nitrate are sometimes assimilated, but *myo*-inositol is not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 is formed. Xylose is absent in whole-cell hydrolyzates.

1.2.2. *Bullera* (including *Udeniomyces*; teleomorph is *Bulleromyces*):

Vegetative reproduction: Colonies are whitish, orange to reddish-brown, and butyrous to mucoid. Yeast cells are subglobose, ellipsoidal to elongate, and usually have polar budding. Hyphae and pseudohyphae may be present. Ballistoconidia are rotationally symmetrical (in *Bullera* sensu stricto) or bilaterally symmetrical (in *Udeniomyces*). Septal pores, if present, are dolipores with cupulate parenthesomes (clamp connections are present in the dikaryophase of *B. variabilis*).

Physiology: Fermentation is absent. D-Glucuronate is assimilated, and *myo*-inositol and nitrate are sometimes assimilated. Starch-like compounds are sometimes produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates. (Note: considerable evidence suggests that *Udeniomyces* represents a separate genus, distinct by bilaterally symmetrical ballistoconidia, presence of collarettes and assimilation of nitrate).

1.2.3. *Cryptococcus* (yeast stages of Tremellales are similar to *Cryptococcus*):

Vegetative reproduction: Colonies are whitish, cream-colored, reddish, yellowish to brown, and usually mucoid. Yeast cells are globose to subglobose, ovoidal to elongate, and with polar or multilateral budding. Hyphae and pseudohyphae may be present. Chlamydospore-like cells and clamp connections may be formed following conjugation. Septal pores, if present, are dolipores without parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate is assimilated, *myo*-inositol is usually assimilated, and nitrate is sometimes assimilated. Starch-like compounds are usually produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-8, 9 or 10 are formed. Xylose is present in whole-cell hydrolyzates.

1.2.4. *Fellomyces*:

Vegetative production: Colonies are cream-colored and butyrous. Yeast cells are globose to subglobose to ellipsoidal, with budding occurring on stalks with the septa at the distal ends. Hyphae and pseudohyphae may be present. Septal pore structure is not determined.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated; nitrate is not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

1.2.5. *Hyalodendron* (possibly synonymous with *Trichosporon*, G.S. de Hoog, personal communication):

Vegetative reproduction: Colonies are whitish to cream-colored and farinose to velvety. Hyphae may disarticulate. Truncate blastoconidia occur in acropetal chains. Chlamydospore-like structures and yeastlike cells may be present. Septal pores are dolipores with cupulate parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate assimilation is not known; *myo*-inositol is assimilated, but nitrate is not assimilated. Production of starch-like compounds is not known. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 is formed. Xylose is present in whole-cell hydrolyzates.

1.2.6. *Itersonilia*:

Vegetative reproduction: Hyphal colonies are whitish, hairy, velvety, arachnoid or velutinous. Hyphae have clamp connections and chlamydospore-like ("sporogenous") cells. Ballistoconidia are bilaterally symmetrical. Yeast colonies are yellowish cream-colored and butyrous. Yeast cells are ellipsoidal to elongate, and usually have polar budding. Hyphae and pseudohyphae may be present. Septal pores are dolipores without parenthesomes.

Physiology: Fermentation is absent. D-Glucuronate is assimilated, *myo*-inositol is sometimes assimilated, and nitrate is usually assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 is formed. Xylose is present in whole-cell hydrolyzates. (*I. perplexans* may represent a cryptic teleomorph).

1.2.7. *Kockovaella*:

Vegetative reproduction: Colonies are grayish-orange to yellow, and butyrous to mucoid. Yeast cells are globose to subglobose, ellipsoidal to kidney-shaped, and with budding on stalks with the septa at the distal end. Ballistoconidia are bilaterally symmetrical. Hyphae and pseudohyphae are absent.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated, but nitrate is not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

1.2.8. *Kurtzmanomyces*:

Vegetative reproduction: Colonies are pinkish and butyrous. Yeast cells are globose to subglobose, ovoidal to cylindrical, and with budding on stalks with the septa at the distal end. Hyphae may be present. Septal pore structure is not known.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are not assimilated; nitrate is assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme

Q-10 is formed. Xylose is absent in whole-cell hydrolyzates.

1.2.9. *Malassezia*:

Vegetative growth: Colonies are pale yellowish-brown and butyrous to rather dry. Yeast cells are globose to subglobose to ellipsoidal, and usually have monopolar budding, but occasionally sympodial proliferation occurs. Septal pores are absent or "micropore"-like. (Note: short hyphae can be present on skin.)

Physiology: Fermentation is absent. D-Glucuronate, *myo*-inositol, nitrate assimilation and production of starch-like compounds are not determined. Coenzyme Q-9 is formed. Xylose is absent in whole-cell hydrolyzates.

1.2.10. *Moniliella*:

Vegetative growth: Colonies initially are cream-colored, but soon become grayish to olivaceous-black, and are smooth to velvety. Hyphae may disarticulate. Acropetal chains of truncate blastoconidia originate from short denticles. Thick-walled, clavate, aseptate setae, chlamydospores and pseudohyphae may be present. Yeast cells are ellipsoidal to subcylindrical. Septal pores are dolipores without parenthesomes.

Physiology: Fermentation is present. D-Glucuronate assimilation is not known; *myo*-inositol is not assimilated, but nitrate is assimilated. Production of starch-like compounds is not known. Coenzyme Q-9 is formed. Xylose is absent from whole-cell hydrolyzates.

1.2.11. *Phaffia* (teleomorph *Xanthophyllomyces*):

Vegetative growth: Colonies are orange to salmon-red, and butyrous. Yeast cells are ellipsoidal. Pseudohyphae and chlamydospores may be present.

Physiology: Fermentation is present. D-Glucuronate is assimilated, but *myo*-inositol and nitrate are not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

1.2.12. *Pseudozyma* (teleomorphs may belong to the Ustilaginales):

Vegetative reproduction: Colonies are whitish, pinkish, orange or brown, and usually dimorphic. Hyphae are present and lack clamp connections. Acropetal chains of fusiform blastoconidia originate from lateral sterigma-like structures. Chlamydospores may be present. Yeast cells are ellipsoidal, ovoidal to cylindrical, and usually have polar budding. Pseudohyphae may be present. Septal pores, if present, are "micropore"-like structures.

Physiology: Fermentation is absent. D-Glucuronate, *myo*-inositol and nitrate are assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is absent in whole-cell hydrolyzates.

1.2.13. *Reniforma*:

Vegetative reproduction: Colonies are white to cream-colored, and waxy or dry. Yeast cells are allantoidal and frequently occur in stacks. Hyphae and pseudohyphae are absent.

Physiology: Fermentation is absent. D-Glucuronate assimilation is not known, and *myo*-inositol and nitrate are not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-7 is formed. Xylose is absent in whole-cell hydrolyzates.

1.2.14. *Rhodotorula* (teleomorphs belong to *Rhodosporidium*):

Vegetative reproduction: Colonies are orange, red, yellow or pale, and butyrous to mucoid. Yeast cells are globose to subglobose, ellipsoidal, ovoidal to elongate, and usually have polar budding. Hyphae and pseudohyphae may be present. Septal pore structure is not known.

Physiology: Fermentation is absent. D-Glucuronate and nitrate are sometimes assimilated, but *myo*-inositol is usually not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 or 10 is formed. Xylose is absent in whole-cell hydrolyzates.

1.2.15. *Sporobolomyces* (including *Ballistosporomyces*; teleomorphs belong to *Sporidiobolus*):

Vegetative reproduction: Colonies are pink, orange, red, yellowish or pale, and butyrous to mucoid. Yeast cells are globose to subglobose, ellipsoidal, fusoidal to cylindrical, and usually have polar budding, but multilateral budding may occur as well. Ballistoconidia are bilaterally symmetrical. Hyphae and pseudohyphae may be present. Septal pore structure is not known.

Physiology: Fermentation is absent. D-Glucuronate and nitrate may or may not be assimilated, and *myo*-inositol is usually not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 or 10 (H_2) are formed. Xylose is absent from whole-cell hydrolyzates.

1.2.16. *Sterigmatomyces*:

Vegetative reproduction: Colonies are white or cream-colored and butyrous. Yeast cells are globose to subglobose to ovoidal, and with budding on stalks with the septum in the mid-region. Hyphae and pseudohyphae may be present. Septal pore structure is not known.

Physiology: Fermentation is absent. D-Glucuronate and nitrate are variably assimilated, but *myo*-inositol is not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 is formed. Xylose is absent from whole-cell hydrolyzates.

1.2.17. *Sympodiomycopsis*:

Vegetative reproduction: Colonies are cream-colored and butyrous to mucoid. Yeast cells are obclavate, obovoid to elongate, with holoblastic-annellidic or holoblastic-sympodial budding. Blastoconidia are formed on short acropetal and sympodially branching chains, or are single. Chlamydospore-like structures may be present.

Hyphae and pseudohyphae are present. Septal pores are "simple".

Physiology: Fermentation is absent. D-Glucuronate assimilation is not known, but *myo*-inositol and nitrate are assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Trace amounts of xylose are present in whole-cell hydrolyzates.

1.2.18. *Tilletiopsis* (teleomorphs may belong to the Tilletiales):

Vegetative reproduction: Colonies are white, cream-colored, pink, yellow to brown, venose or velutinous, and tough to butyrous. Yeast cells, if present, are ellipsoidal to elongate, and have polar budding. Hyphae lack clamp connections. Chlamydospores may be present. Ballistoconidia are bilaterally symmetrical. Septal pores are "micropore"-like.

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are sometimes assimilated, but nitrate is not assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is absent in whole-cell hydrolyzates.

1.2.19. *Trichosporon*:

Vegetative reproduction: Colonies are cream-colored, and moist to dry. Yeast cells, if present, are ellipsoidal to elongate, with polar budding. Hyphae usually disarticulate into arthroconidia. Endospores and chlamydospore-like cells may be present. Septal pores, if present, are dolipores with or without cupulate parenthesomes, but are "micropore"-like in *T. pullulans.*

Physiology: Fermentation is absent. D-Glucuronate and *myo*-inositol are usually assimilated, but nitrate usually is not assimilated. Starch-like compounds are sometimes produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 or 10 are formed. Xylose is present in whole-cell hydrolyzates.

1.2.20. *Trichosporonoides* (probably synonymous with *Moniliella,* G.S. de Hoog, personal communication):

Vegetative reproduction: Colonies are cream-colored, but soon become olivaceous-brown, and smooth or cerebriform. Yeast cells, if present, are ellipsoidal with sympodial proliferation. Hyphae may disarticulate. Blastoconidia occur in acropetal chains. Pseudohyphae may be present. Septal pores are dolipores without parenthesomes.

Physiology: Fermentation is present. D-Glucuronate assimilation is not known; *myo*-inositol is not assimilated, but nitrate is assimilated. Production of starch-like compounds is not known. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 is formed. Xylose is absent in whole-cell hydrolyzates.

1.2.21. *Tsuchiyaea*:

Vegetative reproduction: Colonies are pale and butyrous. Yeast cells are globose to subglobose, ovoidal to cylindrical, with budding on stalks and with the septum in the mid-region.

Physiology: Fermentation is absent; D-Glucuronate and *myo*-inositol are assimilated; nitrate is not assimilated. Starch-like compounds are absent or weakly produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 is formed. Xylose is present in whole-cell hydrolyzates.

2. Key to the heterobasidiomycetous genera with yeast states

1. a Basidiocarps present on natural substrates → 2
   b Basidiocarps absent on natural substrates → 8

2(1). a Basidiocarps synnema-like; basidia non-septate or transversely septate; septa "simple" → 3
   b Basidiocarps tuberculate to cerebriform or foliaceous and gelatinous; basidia longitudinally septate (tremelloid); septa with dolipores → 4

3(2). a Basidia clavate, non-septate, with sessile terminal spores; basidiocarps waxy-arid or subgelatinous; D-glucuronate not assimilated; starch-like compounds produced .... *Chionosphaera*: p. 643
   b Basidia cylindric, transversely septate, with basidiospores laterally budding off; basidiocarps capitate to clavate or stemonitoid; D-glucuronate assimilated; starch-like compounds not produced .... *Agaricostilbum*: p. 639

4(2). a Basidia catenulate; basidiospores passively released, sessile, fusiform to narrowly clavate → 5
   b Basidia virtually all terminal, single (rarely catenulate); basidiospores actively released, sterigmate, usually globose to ovoidal or bullate → 6

5(4). a Basidia developing from successive basal clamp connections of a first terminal basidium, maturing acropetally .... *Fibulobasidium*: p. 710
   b Basidia developing from contiguous hyphal cells, maturing basipetally .... *Sirobasidium*: p. 710

6(4). a Basidiocarps erect, coralloid, occurring in dense aggregates, tough, gelatinous, simple or branched .... *Holtermannia*: p. 711
   b Basidiocarps not erect, irregularly pulvinate to cerebriform or foliaceous, (but lacking in some parasitic forms) → 7

7(6). a H-shaped conidia and/or yeast cells usually present; basidiocarps associated with *Arthrinium* on bamboo and other grasses .... *Trimorphomyces*: p. 717
   b Conidia subglobose to subcylindric, never H-shaped; basidiocarps mainly on woody angiosperms or on fungi or lichens .... *Tremella*: p. 712

8(1). a Teliospores and/or basidia present → 9
   b Teliospores and/or basidia absent (anamorphic taxa) → 19

9(8). a Teliospores present → 10
   b Teliospores absent → 15

10(9). a Teliospores brown and echinulate; only hyphal vegetative cells .... *Tilletiaria*: p. 703
   b Teliospores hyaline and smooth-walled; unicellular vegetative cells usually present → 11

11(10). a Starch-like compounds produced; septa, if perforate, have dolipores without parenthesomes; coenzyme Q-8; xylose present in whole-cell hydrolyzates → 12
b Starch-like compounds not produced; septa, if perforate, with "simple" pores; coenzyme Q-9 or 10; xylose absent in whole-cell hydrolyzates → 13
12(11). a No fermentation; clamp connections sometimes present; maximum temperature of growth above 20°C; colonies orange, salmon, cinnamon or pale .... *Cystofilobasidium*: p. 646
b Fermentation sometimes present; clamp connections absent; maximum temperature of growth below 20°C; colonies pale .... *Mrakia*: p. 676
13(11). a Ballistoconidia present .... *Sporidiobolus*: p. 693
b Ballistoconidia absent → 14
14(13). a Colonies orange or red .... *Rhodosporidium*: p. 678
b Colonies not orange or red .... *Leucosporidium*: p. 670
15(9). a Basidia tremelloid; rotationally symmetrical ballistoconidia present .... *Bulleromyces*: p. 641
b Basidia 1-celled; ballistoconidia absent → 16
16(15). a Starch-like compounds produced; nitrate usually not assimilated; coenzyme Q-9 or 10 → 17
b Starch-like compounds not produced; nitrate assimilated; coenzyme Q-10 (H_2) .... *Erythrobasidium*: p. 654
17(16). a Basidiospores in basipetal chains .... *Filobasidiella*: p. 656
b Basidiospores not in basipetal chains → 18
18(17). a *myo*-Inositol assimilated .... *Filobasidium*: p. 663
b *myo*-Inositol not assimilated .... *Xanthophyllomyces*: p. 718
19(8). a Cells strongly reniform and frequently in stacks .... *Reniforma*: p. 798
b Cells not strongly reniform and not in stacks → 20
20(19). a Conidia (buds) on elongated stalks → 21
b Conidia (buds) not on elongated stalks, but formation of conidia on short denticles may be present → 26
21(20). a *myo*-Inositol assimilated; nitrate not assimilated; xylose present in whole-cell hydrolyzates → 22
b *myo*-Inositol not assimilated; nitrate may be assimilated; xylose absent in whole-cell hydrolyzates → 25
22(21). a Ballistoconidia present .... *Kockovaella*: p. 777
b Ballistoconidia absent → 23
23(22). a Dikaryotic chlamydospores and hyphae present .... *Sterigmatosporidium*: p. 700
b Dikaryotic chlamydospores and hyphae absent → 24
24(23). a Septa at the end of the stalks; coenzyme Q-10 .... *Fellomyces*: p. 768
b Septa in the mid-region of the stalks; coenzyme Q-9 .... *Tsuchiyaea*: p. 878
25(21). a Septa at the end of the stalks; coenzyme Q-10 .... *Kurtzmanomyces*: p. 780
b Septa in the mid-region of the stalks; coenzyme Q-9 .... *Sterigmatomyces*: p. 844
26(20). a Ballistoconidia present → 27
b Ballistoconidia absent → 32
27(26). a Vegetative cells hyphal → 28
b Vegetative cells mainly unicellular → 29
28(27). a Clamp connections and dikaryotic chlamydospores ("sporogenous" cells) present; septal pores are dolipores without parenthesomes; coenzyme Q-9 .... *Itersonilia*: p. 775
b Clamp connections and dikaryotic chlamydospores absent; septal pores, if present, not thickened; coenzyme Q-10 .... *Tilletiopsis*: p. 848
29(27). a Ballistoconidia rotationally symmetrical .... *Bullera*: p. 731
b Ballistoconidia bilaterally symmetrical → 30
30(29). a Ballistoconidia large (up to 18 μm long and 10–14 μm wide); conidia (buds) usually leaving collarettes; colonies not orange or red; D-glucuronate and nitrate usually both assimilated; xylose present in whole-cell hydrolyzates .... *Udeniomyces* (in this treatment incorporated in *Bullera*): p. 731
b Ballistoconidia usually smaller; conidia not leaving collarettes or collarettes not distinct; colonies sometimes orange or red; if ballistoconidia are large and the colonies pale, either D-glucuronate or nitrate is assimilated; xylose absent in whole-cell hydrolyzates → 31
31(30). a Colonies not bright orange or red; coenzyme Q-9 .... *Bensingtonia*: p. 723
b Colonies may be bright orange or red; coenzyme Q-10 or 10 (H_2) .... *Sporobolomyces*: p. 828
32(26). a Budding usually monopolar on a broad base (but occasionally also with sympodial proliferation) .... *Malassezia*: p. 782
b Budding not monopolar on a broad base → 33
33(32). a Septate hyphae conspicuously present → 34
b Septate hyphae usually absent (some hyphal fragments may be present) → 38
34(33). a Arthroconidia dominant; usually no blastoconidia in acropetal chains .... *Trichosporon*: p. 854
b Arthroconidia absent (although disarticulating hyphae may occur); acropetal chains of blastoconidia usually present → 35
35(34). a Fermentation present .... *Moniliella*: p. 785
Trichosporonoides: p. 873
b Fermentation absent → 36
36(35). a Blastoconidia with truncate ends; nitrate not assimilated; septal pore a dolipore with a cupulate parenthesome; coenzyme Q-9 .... *Hyalodendron*: p. 773
b Blastoconidia without truncate ends; nitrate assimilated; septal pore, if present, "simple" or "micropore"-like; coenzyme Q-10 → 37

37(36). a Conidiogenesis usually in acropetal chains; septal pore, if present, a "micropore"-like structure; xylose absent in whole-cell hydrolyzates .... *Pseudozyma*: p. 790

b Conidiogenesis usually with sympodial proliferation; septal pore "simple"; xylose present in trace amounts in whole-cell hydrolyzates .... *Sympodiomycopsis*: p. 846

38(33). a Fermentation present .... *Phaffia*: p. 789

b Fermentation absent → 39

39(38). a *myo*-Inositol usually assimilated; starch-like compounds usually produced; xylose present in whole-cell hydrolyzates .... *Cryptococcus*: p. 742

b *myo*-Inositol usually not assimilated; starch-like compounds not produced; xylose absent in whole-cell hydrolyzates .... *Rhodotorula*: p. 800

Chapter 76

Keys to the genera and species of ballistoconidia-forming yeasts and yeastlike fungi

T. Boekhout

1. Key to the genera of ballistoconidia-forming yeasts and yeastlike fungi

1. a Basidiocarps present on natural substrates → 2

   b Basidiocarps absent on natural substrates → 3

2(1). a Basidia develop from successive basal clamp connections of a first terminal basidium and mature acropetally .... *Fibulobasidium*: p. 710

   b Basidia develop from contiguous hyphal cells and mature basipetally .... *Sirobasidium*: p. 710

3(1). a Clamp connections present; hyphae usually dikaryotic → 4

   b Clamp connections absent; hyphae and yeast cells usually monokaryotic → 6

4(3). a Teliospores present; colonies red, orange-red or pinkish-red; ballistoconidia bilaterally symmetrical .... *Sporidiobolus*: p. 693

   b Teliospores absent; colonies whitish; ballistoconidia bilaterally or rotationally symmetrical → 5

5(4). a Phragmobasidia present; haustorial branches present; ballistoconidia rotationally symmetrical .... *Bulleromyces*: p. 641

   b Phragmobasidia absent; haustorial branches absent; ballistoconidia bilaterally symmetrical .... *Itersonilia*: p. 775

6(3). a Ballistoconidia rotationally symmetrical .... *Bullera*: p. 731

   b Ballistoconidia bilaterally symmetrical → 7

7(6). a Colonies mainly consist of narrow hyphae → 8

   b Colonies mainly consist of yeast cells; monokaryotic hyphae sometimes present → 9

8(5). a Echinulate, brown teliospores present .... *Tilletiaria*: p. 703

   b Echinulate, brown teliospores absent .... *Tilletiopsis*: p. 848

9(7). a Blastoconidia (buds) formed on long stalks .... *Kockovaella*: p. 777

   b Blastoconidia (buds) sessile or on short denticles, only rarely on somewhat elongated stalks → 10

10(9). a Colonies red, orange, orange-red or pinkish-red .... *Sporobolomyces*: p. 828

   b Colonies whitish, cream, brownish-yellow, ochraceous, brown or pale pink → 11

11(10). a Ballistoconidia large (up to 18 µm long and 10–14 µm wide); conidia (buds) usually leave distinct scars forming annellations or collarettes; D-glucuronate and nitrate usually assimilated; xylose present in whole-cell hydrolyzates .... *Bullera* (*Udeniomyces*): p. 731

   b Ballistoconidia usually smaller; conidia (buds) usually do not leave collarettes or collarettes are not distinct; either D-glucuronate or nitrate may be assimilated; xylose absent in whole-cell hydrolyzates → 12

12(11). a Colonies may be bright orange or red; main ubiquinone CoQ-10 or 10(H_2) *Sporobolomyces* (including *Ballistosporomyces*): p. 828

   b Colonies not bright orange or red; main ubiquinone Q 9 .... *Bensingtonia*: p. 723

2. Key to the species with bilaterally symmetrical ballistoconidia

1. a Nitrate assimilated → 2

   b Nitrate not assimilated → 28

2(1). a Sucrose assimilated → 3

   b Sucrose not assimilated → 24

3(2). a Lactose assimilated → 4

   b Lactose not assimilated → 9

4(3). a Raffinose assimilated → 5

   b Raffinose not assimilated → 8

5(4). a *myo*-Inositol assimilated .... *Bullera pyricola*: p. 737

   b *myo*-Inositol not assimilated → 6

6(5). a Galactose assimilated .... *Sporobolomyces xanthus*: p. 841

   b Galactose not assimilated → 7

7(6). a L-Sorbose assimilated .... *Bensingtonia miscanthi*: p. 726

   b L-Sorbose not assimilated .... *Bensingtonia ingoldii*: p. 724

8(4). a Starch assimilated; salicin not assimilated .... *Sporobolomyces lactophilus*: p. 834

   b Starch not assimilated; salicin assimilated .... *Sporobolomyces tsugae*: p. 841

9(3). a Raffinose assimilated → 10

   b Raffinose not assimilated → 20

10(9). a Maltose assimilated → 11

   b Maltose not assimilated → 18

11(10). a Trehalose assimilated → 12

   b Trehalose not assimilated → 17

12(11). a 2-Keto-D-gluconate assimilated → 13
b 2-Keto-D-gluconate not assimilated → 14
13(12). a D-Xylose assimilation .... *Bullera megalospora*: p. 735
b D-Xylose not assimilated .... *Bullera punicea*: p. 739
14(12). a α-Methyl-D-glucoside assimilated → 15
b α-Methyl-D-glucoside not assimilated → 16
15(14). a Colonies salmon-pink or orange-pink; mol% G+C ca. 59–63 .... *Sporobolomyces salmonicolor*: p. 839
b Colonies reddish; mol% G+C ca. 50–56
.... *Sporobolomyces roseus* (compare with rare nitrate assimilating strains of *S. shibatanus*): p. 836
16(14). a Galactitol assimilated; teliospores, dikaryotic hyphae and clamp connections present .... *Sporidiobolus ruineniae*: p. 696
b Galactitol usually not assimilated; teliospores, dikaryotic hyphae and clamp connections absent .. *Sporobolomyces roseus*: p. 836
17(11). a Melibiose assimilated; colonies pale .... *Bensingtonia naganoensis*: p. 726
b Melibiose nor assimilated; colonies reddish .... *Sporobolomyces roseus*: p. 836
18(10). a L-Sorbose assimilated .... *Sporobolomyces salmonicolor*: p. 839
b L-Sorbose not assimilated → 19
19(18). a Cellobiose and D-xylose assimilated; ballistoconidia 12–18 µm long .... *Sporobolomyces salicinus*: p. 838
b Cellobiose and D-xylose not assimilated; ballistoconidia 6–11 µm long .... *Sporobolomyces subbrunneus*: p. 840
20(9). a *myo*-Inositol assimilated .... *Sporobolomyces inositophilus*: p. 833
b *myo*-Inositol not assimilated → 21
21(20). a L-Sorbose assimilated → 22
b L-Sorbose not assimilated .... *Sporobolomyces griseoflavus*: p. 832
22(21). a D-Arabinose, and usually L-arabinose assimilated → 23
b D-Arabinose and L-arabinose not assimilated .... *Bensingtonia phyllada*: p. 727
23(22). a α-Methyl-D-glucoside assimilated; 2-keto-D-gluconate not assimilated .... *Sporobolomyces salmonicolor*: p. 839
b α-Methyl-D-glucoside not assimilated; 2-keto-D-gluconate assimilated .... *Sporobolomyces foliicola*: p. 831
24(2). a Maltose assimilated .... *Sporobolomyces roseus*: p. 836
b Maltose not assimilated → 25
25(24). a Cellobiose assimilated → 26
b Cellobiose not assimilated → 27
26(25). a D-Xylose and D-glucosamine assimilated .... *Sporobolomyces falcatus*: p. 831
b D-Xylose and D-glucosamine not assimilated .... *Bensingtonia yuccicola*: p. 729
27(25). a D-Xylose and 2-keto-D-gluconate assimilated .... *Sporobolomyces kluyveri-nielii*: p. 834
b D-Xylose and 2-keto-D-gluconate not assimilated .... *Bensingtonia ciliata*: p. 724
28(1). a 2-Keto-D-gluconate assimilated → 29
b 2-Keto-D-gluconate not assimilated → 35
29(28). a Galactose assimilated → 30
b Galactose not assimilated → 32
30(29). a Raffinose assimilated; L-rhamnose not assimilated → 31
b Raffinose not assimilated; L-rhamnose assimilated .... *Sporobolomyces phyllomatis*: p. 835
31(30). a Ethanol assimilated; growth at 37°C .... *Sporobolomyces alborubescens*: p. 830
b Ethanol not assimilated; growth absent at 37°C .... *Sporobolomyces oryzicola*: p. 835
32(29). a L-Sorbose and D-ribose assimilated .... *Bensingtonia intermedia*: p. 725
b L-Sorbose and D-ribose not assimilated → 33
33(32). a Sucrose assimilated → 34
b Sucrose not assimilated .... *Sporobolomyces singularis*: p. 839
34(33). a Maltose and trehalose assimilated .... *Bensingtonia yamatoana*: p. 729
b Maltose and trehalose not assimilated .... *Sporobolomyces ruber*: p. 837
35(28). a Sucrose assimilated → 36
b Sucrose not assimilated .... *Sporobolomyces gracilis*: p. 832
36(35). a Melibiose assimilated .... *Sporobolomyces ruber*: p. 837
b Melibiose not assimilated → 37
37(36). a D-Arabinose assimilated → 38
b D-Arabinose not assimilated → 40
38(37). a α-Methyl-D-glucoside assimilated → 39
b α-Methyl-D-glucoside not assimilated .... *Sporobolomyces elongatus*: p. 830
39(38). a Colonies salmon-pink or pinkish-orange; mol% G+C ca. 59–63 .... *Sporobolomyces salmonicolor*: p. 839
b Colonies reddish; mol% G+C ca. 50–56 .... *Sporobolomyces shibatanus*: p. 839
40(37). a Ethanol and glycerol assimilated; L-arabinose not assimilated .... *Bensingtonia subrosea*: p. 728
b Ethanol and glycerol not assimilated; L-arabinose assimilated .... *Sporobolomyces sasicola*: p. 839

Part VIb

Descriptions of teleomorphic basidiomycetous genera and species

Contents

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77. *Agaricostilbum* Wright

R.J. Bandoni and T. Boekhout

Diagnosis of the genus

Basidia occur predominantly in synnemata-like basidiomata, (Fig. 292a, p. 616) but reduced basidiocarps may consist of only a few hyphal strands. The fertile hyphae usually terminate with four-celled basidia (Fig. 293, p. 617) and each basidial cell buds off sporidia. Many of the spores remain attached at any one time. Both the basidia and the basidiospores tend to become thick-walled, perhaps because of the exposed habitats occupied by these fungi.

Little is known of the biology of these fungi. They typically occur on well-weathered palm material with numerous other fungi, but whether or not they are mycoparasites is unknown. The life cycle appears to be as follows: haploid yeast states conjugate and give rise to dikaryotic hyphae without clamp connections; these form synnemata on which the basidia occur; germination of the basidiospores result in the haploid yeast state. Conidial states are not known. Basidiomata of *A. pulcherrimum* and *A. hyphaenes* develop readily in culture. The yeast state of a single species is characterized here.

Systematic discussion of the species

77.1. *Agaricostilbum hyphaenes* (Harrison & Patouillard) Oberwinkler & Bandoni (1982b)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal to fusoidal, occasionally somewhat cylindrical, (4.0–10.0)×(2.0–4.0) μm, and single. Budding is polar with enteroblastic proliferation and usually on a short denticle. Colonies are pale yellowish-cream, smooth, shiny, butyrous, and with the margin entire.

Growth on the surface of assimilation media (glucose): Growth is absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 5 days at 25°C, no hyphae or pseudohyphae are formed, but short filaments may occur. Aerobic growth is pale yellowish-cream, dull, butyrous, flat, smooth or somewhat warty, and with the margin entire. Cells are similar to those on malt extract agar, but frequently form short chains or aggregates.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	l	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	s	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	l	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	s	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: CBS 7811, from basidiomes growing on a palm spathe (*Pheonix canariensis*), collected by W. Schofield, Stanford, California, USA.

Representative strain: CBS 7811.

Comments on the genus

The genus *Agaricostilbum* Wright was erected for a fungus first thought to be a synnematous hyphomycete (Wright 1970). The fungus, *A. palmicolum* Wright, was later found to be a gasteroid heterobasidiomycete (Wright et al. 1981), and was placed in the heterogeneous order, Atractiellales, by Oberwinkler and Bandoni (1982b) with *A. hyphaenes* (Harrison & Patouillard) Oberwinkler & Bandoni as the only other species. *A. palmicolum,* now known as *A. pulcherrimum* (Berkeley & Broome) Brady, Sutton & Samson, was assigned to a new order, Agaricostilbales, based on septal pore structure, discoidal spindle pole bodies (SPB), and morphological features of the basidia and basidiomata (Oberwinkler and Bauer 1989, Bauer et al. 1992).

Although the basidia of *Agaricostilbum* are similar to those of *Ustilago* species, the genus *Agaricostilbum* does not appear to be closely related to any other heterobasidiomycetous groups on the basis of 5S ribosomal RNA sequence comparisons (Gottschalk 1985, Gottschalk and Blanz 1985). Bauer et al. (1992) found that the structure of the SPB was intermediate between those of the smuts and rusts.

Species of *Agaricostilbum* are common on dead palm fronds and inflorescences. *A. pulcherrimum* has also been reported from a species of *Xanthorrhoea.* Representatives of the genus are among the commonest fungi occurring

on palm litter in warmer areas of Australia and parts of Asia (R.J. Bandoni, personal observation) and probable other regions where palms grow. The species are of seasonal occurrence, however, and therefore are not always present in spite of having rather tough, persistent basidiomata.

78. *Bulleromyces* Boekhout & A. Fonseca

T. Boekhout

Diagnosis of the genus

Yeast cells are ellipsoidal, subglobose or cylindroid, and monokaryotic. Budding is polar, with buds enteroblastic, sessile or on short denticles and with percurrent or sympodial proliferation. Ballistoconidia are rotationally symmetrical, subglobose. Colonies are cream, smooth, butyrous or mucoid.

The teleomorphic state may be heterothallic or self-sporulating. Basidiocarps are absent. Dikaryotic hyphae are regularly branched, septate, with clamp connections and haustorial branches. Septal pores are dolipores with cupulate parenthesomes. Basidia are subglobose, clavate or ovoidal, and after karyogamy become longitudinally, obliquely or transversely and two to four-celled septate. Germination of basidia gives yeast cells or hyphae on which yeast cells or ballistospores originate.

Fermentation is absent. D-glucuronate and *myo*-inositol are assimilated, but nitrate is not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

The anamorph is assigned to *Bullera*.

Type species

Bulleromyces albus Boekhout & A. Fonseca

Species accepted

1. *Bulleromyces albus* Boekhout & A. Fonseca

Systematic discussion of the species

78.1. *Bulleromyces albus* Boekhout & A. Fonseca (Boekhout 1991a)

Anamorph: *Bullera alba* (Hanna) Derx
Synonyms:
Sporobolomyces albus Hanna (Bisby et al. 1929)
Bullera alba (Hanna) Derx (1930)

Growth of yeast cells on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal, ovoidal or subglobose, (5.0–11.0)×(3.0–6.0) μm, and single. Budding is polar and buds are sessile or on short denticles. Cells sometimes form hyphal outgrowths of up to ca. 80 μm long and 2.0–5.0 μm wide. Growth is mucoid, flat or slightly raised, smooth, dull or shiny, cream to brownish-cream, and with an entire margin.

Growth on the surface of glucose assimilation medium: A thin film, islets, a ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, hyphae are sometimes formed, ca. 100 μm long and 2.0–4.0 μm wide. Subglobose, somewhat thick-walled cells occur with the outer cell wall layer peeling off. Aerobic growth is cream, pale yellowish, or pale yellowish-brown, shiny, mucoid, smooth or slightly venose, flat, and the margin entire, straight or crenulate.

Formation of ballistoconidia: Ballistoconidia that form on corn meal agar and malt extract agar are ellipsoidal to subglobose, and measure (4.0–9.5)×(3.5–7.0) μm (Fig. 310).

Formation of dikaryophase: Conjugation occurs after mixing of strains of different mating types on corn meal agar at 17°C or at room temperature. Self-sporulating strains also occur. Dikaryotic hyphae are made up of cells measuring (40–130)×(1.5–3.0) μm, with clamp connections and haustorial branches. Basidia occur laterally and terminally on hyphae or hyphal branchlets, and measure (5.0–10.0)×(4.0–7.0) μm, finally becoming 2–4 celled, because of longitudinal, transverse or oblique septation (Fig. 311). Basidia germinate by forming yeast cells or hyphae on which yeast cells or ballistospores are formed.

Fermentation (17°C): absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

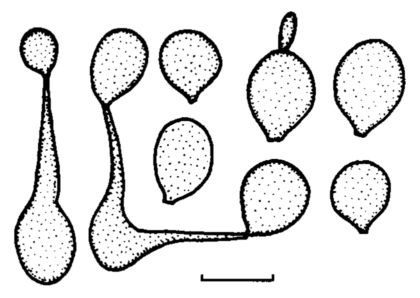


Fig. 310. *B. albus,* CBS 501. Yeast phase. Ballistoconidia on corn meal agar. Bar = 10 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase and Suzuki 1986a).

Mol% G + C: 53.3–54.4, four strains, CBS 500, CBS 501, CBS 502, CBS 6302 (T_m: Boekhout 1991a); 54.5 (T_m: Nakase et al. 1990c); 54.4 (T_m: Nakase and Komagata 1971g).

Origin of the strains studied: CBS 500, type of *Sporobolomyces albus* Hanna, straw of *Hordeum jubatum* infected by rust fungi, W.F. Hanna, USA; CBS 501, air in dairy, B.W. Hammer, USA; CBS 502, air in dairy, B.W. Hammer, USA; CBS 6097, frass of *Sinoxylon ruficorne,* J.P. van der Walt, South Africa; CBS 6302, grass, P.F. Dupont, USA; CBS 7440, leaf of poplar (*Populus* sp.), A. Fonseca, Portugal; CBS 7441, leaf of poplar (*Populus* sp.), A. Fonseca, Portugal; CBS 7503, leaf of walnut (*Juglans* sp.), G. Giménez, Portugal.

Complementary mating types: CBS 500 (mt *A*), CBS 7441 (mt *A*), CBS 7503 (mt *A*), CBS 501 (mt *B*), CBS 6097 (mt *B*), CBS 6302 (mt *B*).

Type strain: CBS 500 × CBS 6302 (herbarium, CBS).

Comments on the genus

Lodder and Kreger-van Rij (1952) designated strain No 16, isolated by Hammer as neotype for *Bullera alba.* However, CBS 500, originally isolated by Hanna, and the type strain of *Sporobolomyces albus,* is the original type strain of *Bullera alba.*

Bullera alba var. *lactis* was found to be conspecific with *Bullera sinensis* (Nakase et al. 1990c, Boekhout 1991a). As a consequence, no mating reactions occurred between this species and the monokaryophase of *Bulleromyces albus.* The monokaryophase of *B. albus* (= *Bullera alba*) can be differentiated from related species by assimilation of inulin. In addition, the frequent assimilation of L-sorbose, D-glucosamine, and erythritol, and lack of growth with nitrite may be used to differentiate this species.

Bandoni (1987) reported the occurrence of mating and formation of dikaryotic hyphae with clamp connections. Boekhout et al. (1991a) observed phragmobasidia of the *Tremella*-type after mating strains of *Bullera alba.* The septal pore is a dolipore with a cupulate parenthesome (Tremellales type), which supports the proposed relationship of *Bullera* with the Tremellales (Phaff 1970d, Boekhout et al. 1991a).

Formation of dikaryotic hyphae and probasidium-like cells was also observed among strains of *Bullera variabilis* (Boekhout et al. 1991a, Boekhout 1991a). However, in this case no development of basidia was observed.

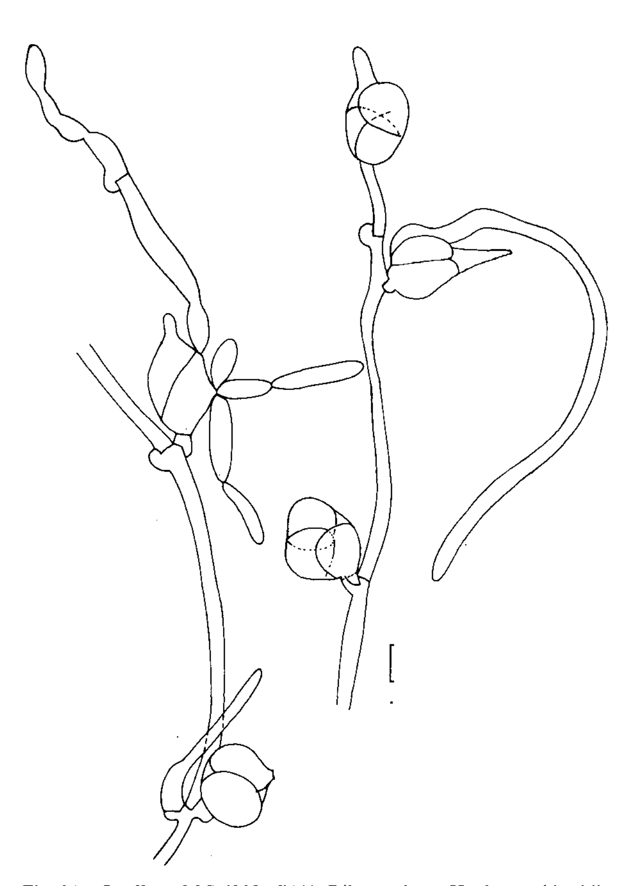


Fig. 311. *B. albus,* CBS 6302 × 7441. Dikaryophase. Hyphae and basidia on corn meal agar. Bar = 10 μm.

79. *Chionosphaera* Cox

K.J. Kwon-Chung

Diagnosis of the genus

Asexual reproduction is by polar budding of ellipsoidal to sausage-shaped single cells (Fig. 312A). Basidiocarps are white, minute, stipitate-capitate, and abundant (Fig. 312B). The stipes are composed of bundles of parallel, infrequently septate hyphae with no clamp connections (Fig. 312C, 313A1). Septal pores are simple. Basidial caps are often waxy, but sometimes cottony, globose to slightly flattened, without hyphidia or other sterile elements (Fig. 312B). Basidia are clavate, terminal, and produced mostly on unilaterally proliferating hyphal branches (Fig. 312C). Basidiospores are hyaline, oblong to ellipsoidal or reniform, one-celled, smooth (Fig. 312D), six to eight in number, and produced on short sterigmata in tight apical clusters (Fig. 313A3). After the basidiospores are released, the basidial apex is crown-like, due to the plugged short sterigmata (Fig. 313A4).

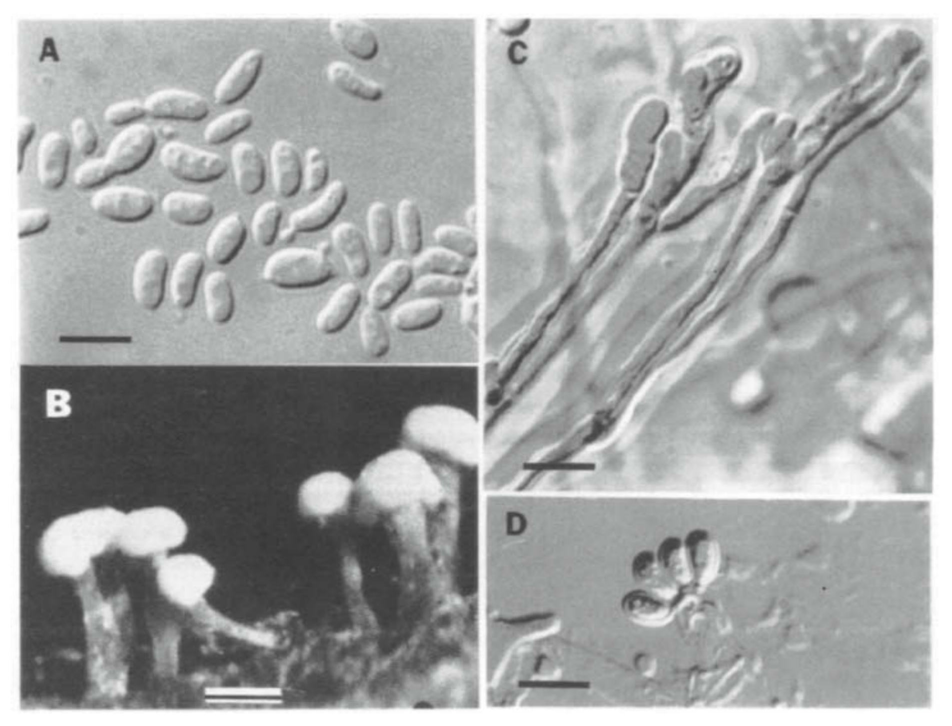


Fig. 312. *C. apobasidialis.* (A) Yeast cells grown on malt agar. Bar = 10 µm. (B) Mature basidiocarps on dead limbs of *Carpinus.* Bar = 0.3 mm (Cox 1976). (C) Young basidia produced terminally on septate hyphae. Bar = 10 µm. (D) Ovoidal to reniform basidiospores. Bar = 10 µm.

Fermentation is absent. D-glucuronate, *myo*-inositol and nitrate are not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Cell wall composition is not determined.

Type species

Chionosphaera apobasidialis Cox

Species accepted

1. *Chionosphaera apobasidialis* Cox (1976)

Systematic discussion of the species

79.1. *Chionosphaera apobasidialis* Cox (1976)

Growth on yeast extract–2% malt agar: After 3 days at 25°C, the cells are ellipsoidal to sausage-shaped, (1.5–3)×(3–6) μm, and are single or in pairs (Fig. 312A). The colony is slightly mucoid and cream-colored. Dikaryotic mycelium is formed if the isolates are self-fertile.

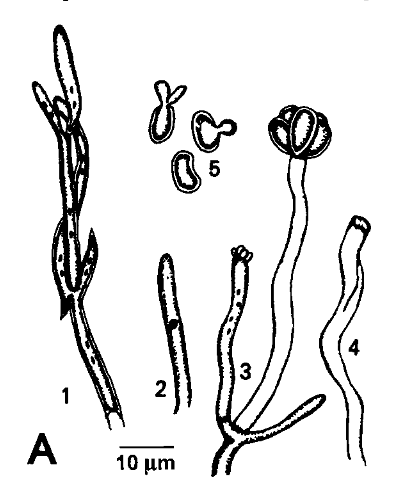
Growth on the surface of assimilation media (yeast nitrogen base plus glucose): No pellicles, but rings are formed.

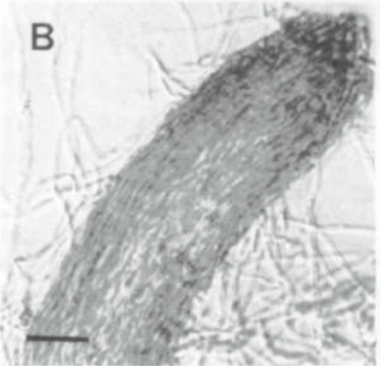
Morphology on corn meal agar: After 3 days at 25°C, the cells are ovoid, ellipsoidal to sausage-shaped, measuring (1.2–4)×(3–7) μm, and are single or in pairs. A limited amount of pseudohyphae is produced in old cultures.

Life cycle: Yeast cells of the two opposite mating types fuse by a conjugation tube, and the contents of one of the fused cells flows into the other cell via the conjugation tube. Dikaryotic hyphae without clamp connections arise from the second cell and eventually produce white, erect, capitate fascicles. The fascicles are composed of compact, predominantly parallel, infrequently branched, and infrequently septate hyphae which form synnemata (Fig. 313B). The synnemata are terminated with a globose sporocarp (0.25–0.75 mm) covered with a hymenium (Fig. 313C). The hymenium is composed of frequently-branched hyphae bearing terminal basidia (Fig. 312C). Basidia, (20–49×3–5) μm, are evenly or undulately clavate, bearing six to eight thick-walled basidiospores apically on short denticles (sterigmata). The sterigmata collapse after the spores are detached. Basidiospores, (3.5–5×7–10) μm, are hyaline, smooth, ellipsoidal to ovoidal, or reniform. Basidiospores germinate to produce uninucleate yeast cells.

Two isolates, CBS 7430 and CBS 7431, were both self-fertile and produced basidiocarps when the yeast cells were initially grown on yeast extract–malt (2%) agar for the formation of dikaryotic mycelium followed by transfer of the mycelial blocks onto water agar. Single basidiospores isolated from CBS 7430 produced two kinds of isolates: self-fertile and self-sterile. Crossing of self-sterile isolates in all possible combinations revealed that they belong to two different mating types. It appears, therefore, that *C. apobasidialis* has bipolar heterothallism, as Cox (1976) has suggested. The isolates lose the ability to form basidiocarps when they are maintained on agar media. The species descriptions of *C. apobasidialis* by Cox (1976), and subsequently by Oberwinkler and Bandoni (1982b), are based on the basidiocarps produced in its natural habitat, the bark of *Quercus* and *Carpinus* trees. The basidiocarps, (0.5–3 mm high), produced on the tree bark resemble minute mushrooms with white, globose to depressed globose caps, often waxy, and with short stipes (Fig. 312B). Such well-developed basidiocarps have been rarely observed in pure culture on agar media. Cox (1976) has reported that, in the presence of dematiaceous hyphomycetes such as *Cladosporium* or *Chloridium,* fasciculate, globose basidiocarps are produced by *C. apobasidialis* on agar medium. Most of the basidiocarps produced in pure culture were loosely fasciculated, bearing a scanty hymenium at their tips.

Fermentation: absent.





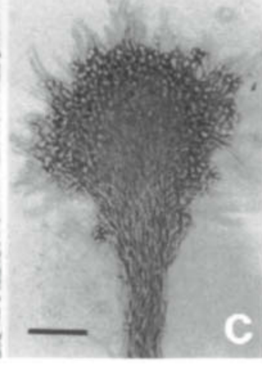


Fig. 313. *C. apobasidialis.* (A1) Dikaryotic hyphae and early stages of basidium formation. (A2) Diploidization in young basidium. (A3) Basidia with basidiospores. (A4) Basidium after release of basidiospores. (A5) Germinating basidiospores. (B) Synnemata. Bar = 10 μm. (C) Young basidiocarp (Cox 1976). Bar = 80 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	w/–
Urease	w/–		

Co-Q: 10 (Y. Yamada, personal communication).

Mol% G + C: 51.6 (HPLC: P.A. Blanz, personal communication).

Origin of the strains studied: CBS 7430 (NRRL Y-17229) and CBS 7431 (NRRL Y-17230) isolated by Clark T. Rogerson from twigs of *Carpinus caroliniana*. Other strains of *C. apobasidialis* have been isolated from deciduous trees such as *Quercus macrocarpa, Q. stellata,* and *Carpinus caroliniana* (Oberwinkler and Bandoni 1982b, Cox 1976).

Type strain: CBS 7430, a self-fertile strain received from Clark Rogerson, was collected in Chester County, Pennsylvania, USA.

Comments on the genus

The genus *Chionosphaera* was described by Cox (1976) to accommodate a yeast which produces minute, stipitate-capitate basidiocarps resembling those of the homobasidiomycetes. The genus *Chionosphaera* is monotypic. In spite of the presence of basidiocarps, Cox classified *C. apobasidialis* in the family Filobasidiaceae because the basidia are long, nonseptate, and produce apical basidiospores in a flower-like arrangement, characteristics shared by members of the Filobasidiaceae. Cox's isolates were not preserved for further study, and the description of *C. apobasidialis* appearing in the previous edition of this book was based solely on Cox's report. He also proposed to classify Filobasidiaceae in the order Aphyllophorales of the Homobasidiomycetes on the basis that the family produces holobasidia. In 1982, Oberwinkler and Bandoni made a critical study on newly isolated cultures of *C. apobasidialis* (isolated by C. Rogerson) and the herbarium specimen on *Quercus stellata* (collected by Cox). They concluded that *C. apobasidialis* does not fit into the family Filobasidiaceae, and transferred it into the newly described family, Chionosphaeriaceae. Unlike members of the Filobasidiaceae, *C. apobasidialis* produces basidiocarps, and basidiospores are formed on the sterigmata. The hyphal septa in *C. apobasidialis* are simple and resemble those of rust fungi (Oberwinkler and Bandoni 1982b). Hyphal septa of the Filobasidiaceae have primitive to well-developed dolipores without parenthesomes (Kwon-Chung and Popkin 1976, Moore and Kreger-van Rij 1972, Oberwinkler et al. 1983). Physiologically, *C. apobasidialis* differs from the members of the Filobasidiaceae by not being able to assimilate inositol or to produce a strong urease reaction. Nucleotide sequence analysis of the 5′ portion of LSU rDNA (J. Fell, personal communication) demonstrated that *C. apobasidialis* is a Uredinomycete and possibly a member of the Agaricostilbales.

80. *Cystofilobasidium* Oberwinkler & Bandoni

K.J. Kwon-Chung

Diagnosis of the genus

Species of the genus *Cystofilobasidium* except *C. lari-marini,* produce bright orange, pinkish-salmon to cinnamon-colored colonies due to the presence of carotenoid pigments. Asexual reproduction is by polar budding of haploid or diploid, ovoidal to elongated cells. Ballistoconidia are not formed. Rudimentary pseudohyphae may be produced in some species. True mycelium with or without clamp connection is produced during sexual reproduction. Mycelial septa have dolipores without parenthesomes.

In the teleomorphic state, intercalary, globose, thick-walled dark pigmented probasidia (teliospores) are produced on the mycelium. Probasidia germinate to form slender, tubular or short, pyriform holometabasidia with non-deciduous terminal, and sessile basidiospores. Species may be homothallic or heterothallic.

Fermentation may be present. D-Glucuronate, *myo*-inositol and nitrate are sometimes assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 or 10 is formed. Xylose is present in whole-cell hydrolyzates.

Type species

Cystofilobasidium capitatum (Fell, I.L. Hunter & Tallman) Oberwinkler & Bandoni

Species accepted

1. *Cystofilobasidium bisporidii* (Fell, I.L. Hunter & Tallman) Oberwinkler & Bandoni (1983)
2. *Cystofilobasidium capitatum* (Fell, I.L. Hunter & Tallman) Oberwinkler & Bandoni (1983)
3. *Cystofilobasidium infirmominiatum* (Fell, I.L. Hunter & Tallman) Hamamoto, Sugiyama & Komagata (1988)
4. *Cystofilobasidium lari-marini* (Saëz & T.L. Nguyen) Fell & Statzell-Tallman (1992)

Key to species

See Table 67.

1.	a Colony white	*C. lari-marini*:	p. 652
	b Colony pinkish-salmon, or bright orange to cinnamon-colored → 2		
2(1).	a Metabasidium short and pyriform	*C. infirmominiatum*:	p. 650
	b. Metabasidium tubular and slender with a terminal swelling → 3		
3(2).	a Melibiose assimilated	*C. bisporidii*:	p. 646
	b Melibiose not assimilated	*C. capitatum*:	p. 648

Table 67
Key characteristics of species in the genus *Cystofilobasidium*

Species	Colony color	Glucose fermentation	Assimilation[a]				Shape of metabasidia	Sexuality
			Me	Mz	I	LS		
Cystofilobasidium bisporidii	Orange, pink	–	+	+	+	+	Tubular with terminal swelling	Heterothallic
C. capitatum	Orange, pink, cinnamon	–	–	+	+	+	Tubular with terminal swelling	Homothallic(?)
C. infirmominiatum	Orange, pink	–	–	+	+	–	Pyriform	Heterothallic
C. lari-marini	White	s	–	+	+	+	Tubular with terminal swelling	Homothallic

[a] Abbreviations: Me, melibiose; Mz, melezitose; I, inositol; LS, L-sorbose.

Systematic discussion of the species

80.1. *Cystofilobasidium bisporidii* (Fell, I.L. Hunter & Tallman) Oberwinkler & Bandoni (Oberwinkler et al. 1983)

Synonym:
Rhodosporidium bisporidii Fell, I.L. Hunter & Tallman (1973)

Growth in 2% malt extract broth: Haploid cells grown for 3 days at 12°C are subglobose to elongate, (3–5)×(5–11) μm, and usually single or in pairs. Reproduction is by polar budding, and there is a light sediment. A light pellicle and a moderate sediment are present after one month.

Growth on 2% malt agar: After 3 days at 12°C, the colony is orangish-pink, smooth, glistening, and the margin is entire. The yeast cell morphology is subglobose to elongate resembling in size those cells grown in malt broth (Fig. 314A). After one month, the color is the same as at 3 days and the colony is mucoid and slightly runny.

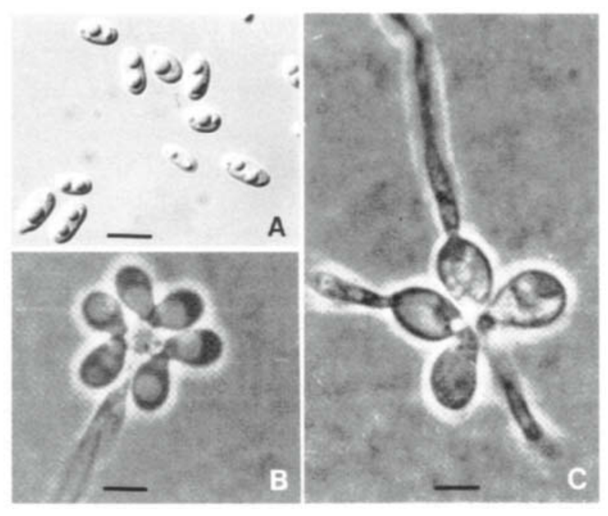


Fig. 314. *C. bisporidii.* (A) Yeast cells grown on 2% malt extract agar. Bar = 10 µm. (B) Basidium with paired primary sporidia. Bar = 5 µm. (Fell et al. 1973). (C) Germ tube formation from the primary sporidia. Bar = 4 µm. (Fell et al. 1973).

At 24°C, the cell morphology is the same as at 12°C, except that brown areas containing globose (5–11 µm in diameter) to subglobose (7–9.5)×(8–10) µm, thick-walled, granulated cells develop within one month of growth. The granulated cells differ from teliospores by thinner walls.

Dalmau plate cultures on corn meal agar: After incubation for 2 weeks at 12°C or at 24°C, a true mycelium or typical pseudomycelium is absent. Occasional rudimentary pseudohyphae or short chains of cells may be present.

Life cycle (Fig. 315): The yeast cells (48 h stock culture) of the two compatible mating types (*A1B1*×*A2B2* or *A2B1*×*A1B2*) conjugate within 4 days when they are mixed on corn meal agar at 12°C. The results are not always dependable, and the procedure may have to be repeated before the mating reaction can be observed. Dikaryotic hyphae with hook-to-peg clamp connections develop from conjugated cells within 4 days. Conidia are absent on the mycelium. The teliospores are globose (9–11 µm in diameter) to subglobose (6–9)×(7–11) µm, uninucleate, granulated, intercalary, and are produced singly or, infrequently, in pairs. The hook-to-peg clamp connections are present between the teliospores and the mycelium. The teliospores increase in size to (8–12)×(9–114) µm after one month. Teliospores germinate at 12–24°C when 2–3-week-old cultures are soaked in distilled water for 8 to 10 weeks and then transferred onto 2% water agar plates. After 3 days on 2% water agar, the teliospores produce a long metabasidium, (1–2)×(19–140) µm, with 0 to 3 septa and a 2–4 µm-wide terminal swelling. Subglobose to ovoidal (3–4)×(4–8) µm primary sporidia (up to 10 in number) develop at or near the terminal swelling; they are attached to the apex of the basidium either individually or in pairs (Fig. 314B). For some metabasidia, primary sporidia may be found along the sides of the basidia. One to several basidiospores are produced from the primary sporidia which remain attached to the metabasidium throughout the basidiospore development. Basidiospores detach from the primary sporidia and reproduce by polar budding to form yeast colonies of four different mating types. Some primary sporidia may produce germ-tubes rather than basidiospores (Fig. 314C). In rare cases, the metabasidium may be branched and septate with two "basidial heads."

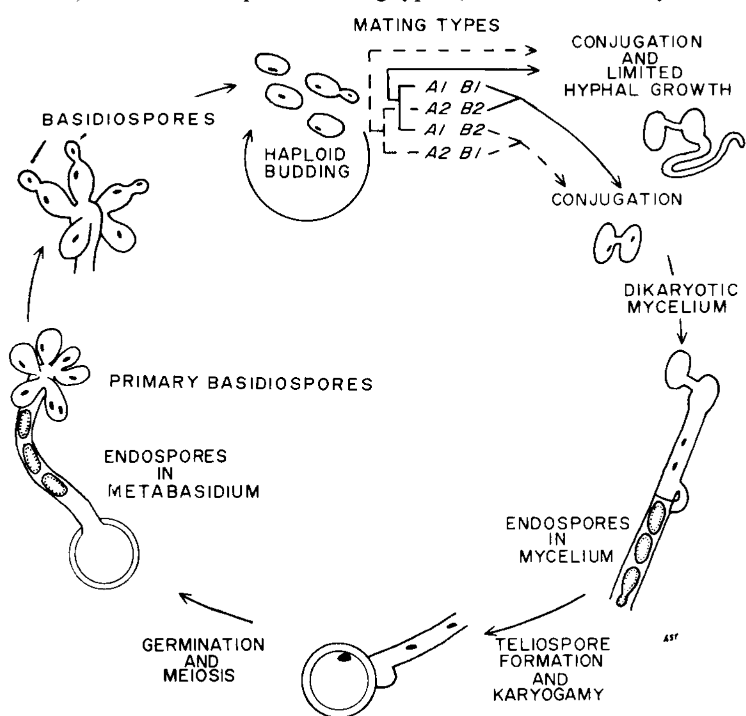


Fig. 315. Life cycle of *C. bisporidii* (Fell and Statzell-Tallman 1984).

Metabasidia occasionally develop directly from thick-walled hyphae in the absence of a teliospore. This indicates that karyogamy can occur in the hyphae and in the teliospore. Endospores (1–6 in number) may be present in the metabasidium or in the mycelium.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	–	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+/w
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	+
D-Ribose	+/w	Hexadecane	v
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

D-Saccharate	–	Thiamine-free	–
Splitting of arbutin	+	Biotin-free	–
50% Glucose (w/w)–yeast extract agar	–	Growth at 24°C	+
		Growth at 30°C	–
Starch formation	–		
Urease	+		

Co-Q: 8 (Yamada and Matsumoto 1988a).
Mol% G+C: 60.9 (T_m: Oberwinkler et al. 1983); 64.1 (HPLC: Hamamoto et al. 1986b).

Origins of the strains studied: Marine sources of the Southern Indian and Pacific Oceans (9) (Fell et al. 1973).

Complementary mating types: CBS 6346 (*A1B1*) and CBS 6347 (*A2B2*).

Type strain: CBS 6346.

Allotype strains: CBS 6348 (*A1B2*) and CBS 6349 (*A2B1*).

Comments: *Cystofilobasidium bisporidii* is the only known species in the family Filobasidiaceae that has a tetrapolar mating system. Only two mating types, *A1B1* and *A2B2,* have been isolated from marine sources, but single-basidiospore cultures of *A2B1* and *A1B2* have been obtained from a parental cross (Fell et al. 1973). Mixes of *A1B1*×*A1B2* or *A2B2*×*A2B1* result in conjugation and deformed hyphae but no clamp connections or teliospores. Mixes of *A1B2*×*A2B2* or *A2B1*×*A1B1,* however, fail to show any indication of mating reactions (Fell et al. 1973). Metabasidia of *C. bisporidii* resemble more closely those of *C. capitatum* than any other *Cystofilobasidium* species. *C. bisporidii,* however, produces metabasidia with considerably smaller apices than *C. capitatum*.

80.2. *Cystofilobasidium capitatum* (Fell, I.L. Hunter & Tallman) Oberwinkler & Bandoni (Oberwinkler et al. 1983)

Synonym:
Rhodosporidium capitatum Fell, I.L. Hunter & Tallman (1973)

Growth in 2% malt extract broth: After 3 days at 12°C, the cells are subglobose to ovoidal or elongate, (1–5)×(2–14) μm, and with or without polar buds. The sediment is light; a ring or pellicle is absent. At one month, a moderate sediment and light film-like ring are present, but without pellicle. Yeast cells grown at 24°C resemble those grown at 12°C, but occasional mycelium and immature teliospores are seen at 24°C. After one month at 24°C, heavy sediment and mycelial clumps are present; a ring may be absent or present, and a pellicle is not produced.

Growth on 2% malt agar: Colonies grown on malt extract agar at 12°C for 3 days are mucoid and light salmon-orange, pinkish, or orange-cinnamon in color. The morphology of cells is similar to that of those grown in malt extract broth (Fig. 316A). At one week, septate true mycelium with terminal, lateral or intercalary teliospores is produced sparsely or abundantly. Conidia are absent on the mycelium. After one month, the colony is deeper in color and usually very mucoid and smooth, although some strains may be convoluted. The colony margin is entire, with areas of dense radiating mycelium.

The teliospores are brown, thick-walled, globose (6–20 μm in diameter) to subglobose (5.9–6.5)×(17.4–18.8) μm, and are produced single, double (Fig. 316B), and in multiples up to 12 or more. After one month, there is some increase in number and size of the teliospores.

At 24°C, colonies are of various shades of cinnamon, light to dark pink, orange, or vinaceous-cinnamon with dark brown sections of teliospores. Colony texture may be rugose and dry to smooth, semi-glistening, or glistening with areas of dense mycelium at the margin. The shapes of

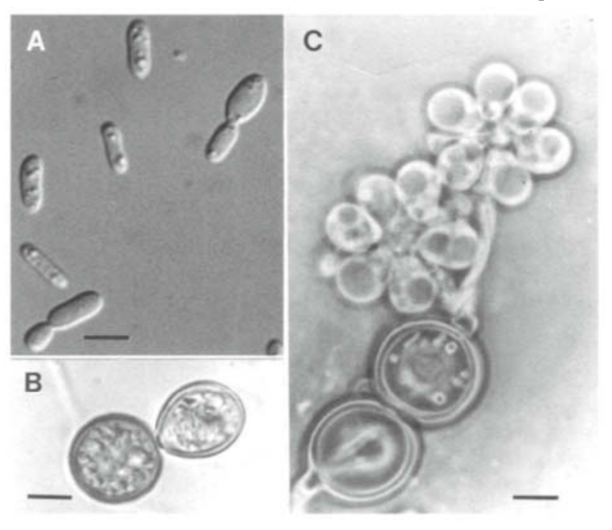


Fig. 316. *C. capitatum.* (A) Yeast cells grown on malt agar. Bar = 10 μm. (B) A pair of teliospores. Bar = 5 μm. (C) Metabasidia with basidiospores developed from a teliospore. Bar = 4 μm. (Fell et al. 1973).

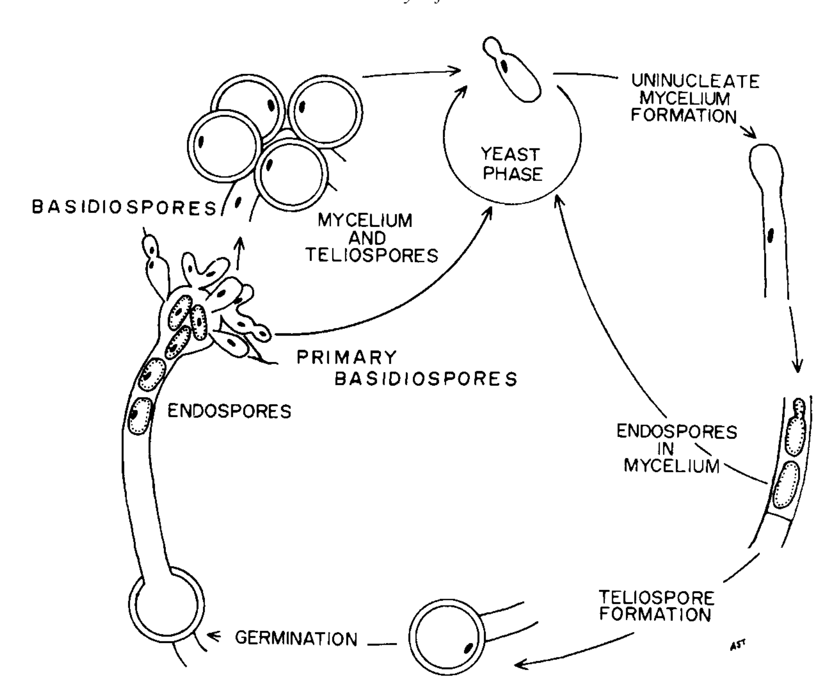


Fig. 317. Life cycle of *C. capitatum* (Fell and Statzell-Tallman 1984).

cells in three-day-old culture varies from ovoidal, subglobose, reniform, dumbbell, elongate, and branched with the size range of 1.3×4.7 μm to 3.4×16 μm. Formation of globose (6 to 8 μm in diameter) to subglobose (5.9×6.5 μm to 17.4×18.8 μm) teliospores become evident at one week, but the size of the teliospores is smaller than those at 12°C. The teliospores are produced on a short mycelium developed from the yeast cells and then multiply by yeastlike budding to form a large group of teliospores. Newly formed teliospores may remain attached to the parent spore and continue to replicate. After one month, colonies become darker in color, ranging from flesh-ocher to ochreous-buff or cinnamon-buff. Teliospores are larger in size, measuring 6.7 to 13.4 μm or 6.7×10 μm to 14.1×14.7 μm. Endospores are absent in the cultures grown at 24°C.

Dalmau plate culture on corn meal agar: Extensive true mycelium with brown-walled teliospores are produced after incubation for 7 days at 12°C and 24°C. Teliospores are globose (4.7 to 15.4 μm in diameter) to ovoidal (5.4×10.8 μm to 6.7×13.4 μm) and are produced single, double, or in groups of 12 or more intercalary, terminally, or laterally on mycelium. Young developing teliospores (up to 7.4×10.1 μm), lacking heavy cell walls and the large globuloid inclusions of the developed spores, are also present. Conidia are lacking. After one month, the teliospores show a slight increase in size. Basal projections (2.2–2.7×1.1 μm) that attach to the mycelium have been seen in some teliospores. Endospores, usually fewer than 6 per mycelial cell, are numerous in one-month-old culture, but rare in 7-day-old culture.

Life cycle (Fig. 317): Single yeast cells produce uninucleate hyphae and teliospores without mating. Clamp connections are absent, but dolipore septa without parenthesomes are present (Oberwinkler et al. 1983). Teliospores do not germinate unless they are first soaked in water for a prolonged time. To germinate teliospores obtained from 3-week-old malt agar culture, the spores are soaked in distilled water for 11 weeks and then transferred onto water agar at 12°C. Germination can be observed after incubation for 3 days. The metabasidium (Fig. 316C), which is rarely septate, is tubular (8–80 μm×1.4–2.7 μm), with an apical swelling, and forms a capitate terminus (4.9–7.7 μm wide). Primary sporidia ranging from 2 to 10 in number develop on the capitate terminus. Primary sporidia are single or in pairs, subglobose (4.0×4.7 μm to 6.0×7.4 μm) with one to two basidiospores, or they may produce septate hyphae (germ tubes). In addition to these teliospores, there are considerably smaller teliospores (2.7×4.0 μm–5.4×6.7 μm) which germinate to produce short (6.0–17.4 μm) basidia with rarely more than two primary sporidia.

After development of basidiospores or germ tubes, primary sporidia degenerate and remain attached to the basidium. Endospores may be formed within the degenerated teliospores and basidium after the completion of basidiospore formation. A metabasidium with primary sporidia may also arise directly from mycelium, which becomes thick-walled. Such a metabasidium is different from those developed from teliospores by lacking a terminal swelling.

Although cytological studies have not clearly determined whether *C. capitatum* is homothallic or the fruiting body is a result of asexual development, all the basidiospore progeny tested were self-fertile. Evidence of genetic recombination was present among the progeny obtained by micromanipulation. Some progeny

were morphologically distinct from the parent culture and produced rugose, dry colonies composed mainly of teliospores, while others were yeastlike in varying degrees. The rugose, dry isolates produced yeast cells only after several weeks in culture.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	+
Lactose	+/w	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	v	D-Gluconate	+
Soluble starch	v	DL-Lactate	+/w
D-Xylose	+	Succinate	–
L-Arabinose	v	Citrate	–
D-Arabinose	+	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

D-Saccharate	–	Thiamine-free	–
Splitting of arbutin	–	Biotin-free	–
50% Glucose (w/w)–yeast extract agar	–	Growth at 24°C	+
		Growth at 30°C	–
Starch formation	+		
Urease	+		

Co-Q: 8 (Sugiyama et al. 1985).

Mol% G+C: 56.6 (T_m: Oberwinkler et al. 1983); 59.2 (HPLC: Hamamoto et al. (1986b).

Origins of the strains studied: Marine sources of the Southern Indian Ocean (13) (Fell 1976) and South Florida (1) (Ahearn and Roth 1966).

Type strain: CBS 6358, isolated from a zooplankton sample in the Southern Indian Ocean.

Comments: The cytology and genetics of *C. capitatum* are extremely difficult to study because the germination of teliospores not only requires a long time and unusual measures (such as soaking in distilled water), but also the rate of germination is low. Oberwinkler et al. (1983) reported that the teliospores can be stimulated to germinate by heating the culture to about 55°C. The details on the growth medium or the length of heating time have not been discussed.

Since the hyphae and teliospores develop directly from a single uninucleate yeast cell, the hyphae are uninucleate with no clamp connections. There is no evidence of nuclear fusion during teliospore formation, and single-basidiospore cultures are self-fertile. Fell et al. (1973) described *C. capitatum* as "self-sporulating" instead of homothallic (Fell and Statzell-Tallman 1984). Because the single-basidiospore cultures vary in morphology and some of them are different from the parent culture, it is likely that recombination and segregation of the factors controlling colonial morphology had occurred during basidiospore formation. Although conclusive evidence has not been obtained, *C. capitatum* may be naturally diploid and homothallic. A question remains as to when and where the reduction division and subsequent diploidization occur in the life cycle. *C. capitatum* was originally isolated from marine sources, but it also occurs on the gleba of mushrooms such as *Mutinus caninus* and *Phallus impudicus* (Oberwinkler et al. 1983).

80.3. *Cystofilobasidium infirmominiatum* (Fell, I.L. Hunter & Tallman) Hamamoto, Sugiyama & Komagata (1988b)

Anamorph: *Cryptococcus infirmo-miniatus* (Okunuki) Phaff & Fell

Synonyms:

Rhodosporidium infirmo-miniatus Fell, I.L. Hunter & Tallman (1973)
Torula infirmo-miniata Okunuki (1931)
Rhodotorula glutinis (Fresenius) F.H. Harrison var. *infirmo-miniata* (Okunuki) Lodder (1934)
Rhodotorula infirmo-miniata (Okunuki) Hasegawa & Banno (1964)
Cryptococcus infirmo-miniatus (Okunuki) Phaff & Fell (1970)
Rhodotorula sinensis M.-H. Lee (1974)

Growth in 2% malt extract broth: After 3 days at 12°C, the cells are subglobose (2.0×3.4)–(4.7×8.7) μm or elongate (1.3×4.0)–(4.7×10) μm, and single or in short chains. Sediment is light, and a ring or pellicle is absent. After one month, a fragile film and ring have formed.

Growth on 2% malt extract agar: After 3 days at 12°C, the colony is grenadine-pink in color, smooth, glistening, and slightly raised. The border is entire without mycelium. The cells are subglobose to elongate (2.0×3.4)–(4.7×8.7) μm. After one month, the colony tends to be mucoid and runny. Some strains are dull, dry, and their margin is lighter in color. The cells are oval to elongate (1–5.5)×(2.5–12) μm or subglobose (2–5.5)×(3–7.5) μm (Fig. 318A). There are globose (4–8 μm in diameter) to subglobose (2–6)×(3–7.5) μm granulated cells with thicker walls than the typical yeast cells.

Dalmau plate culture on corn meal agar: Growth under the coverglass shows abundant yeast cells but no mycelium or pseudomycelium is present at 12°C.

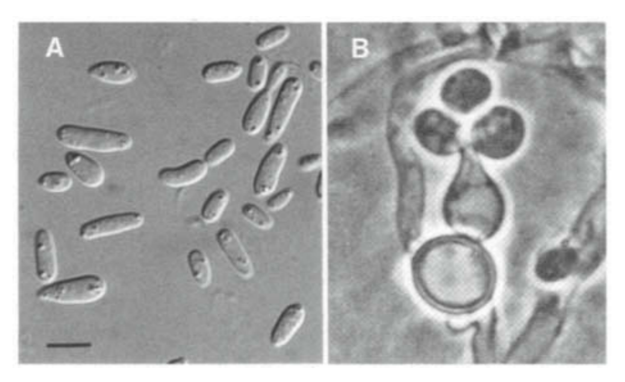


Fig. 318. *C. infirmoniniatum.* (A) Yeast cells grown on malt agar. Bar = 10 μm. (B) Basidium with three basidiospores. Bar = 5 μm. (Fell et al. 1973).

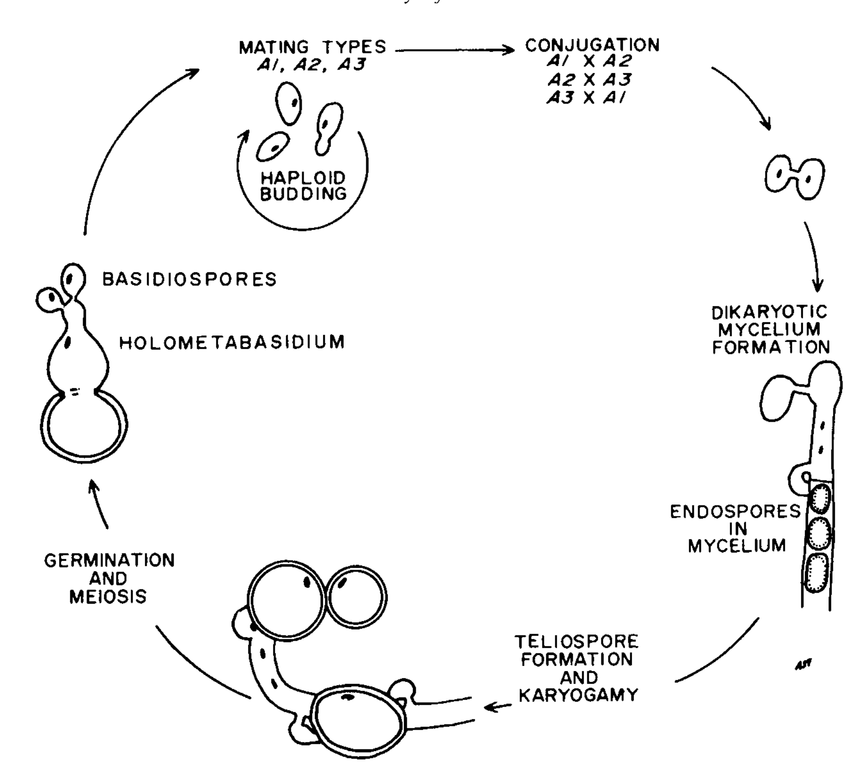


Fig. 319. Life cycle of *C. infirmominiatum* (Fell and Statzell-Tallman 1984).

Life cycle (Fig. 319): *C. infirmominiatum* is a heterothallic species with a one-locus, multiple-allele system. The mating types known are *A1, A2,* and *A3,* and crosses of *A1*×*A2* or *A1*×*A3* produce the same result as the cross of *A2*×*A3*. Upon mixture of two compatible strains on corn meal agar, conjugation occurs within 36 hours and mycelial formation becomes evident after 3 days. After one week, mycelium and teliospores are abundant, although there is some strain variation in their rate of formation and degree of abundance. Clamp connections are of the hook-to-peg type, and they are infrequently found. Conidia are rarely produced on the hyphae. Teliospores are found in two morphological forms: the first type is globose (6.5–8 µm in diameter) to subglobose (5–9)×(7–11) µm with numerous granular bodies and is terminal or intercalary and single or double. Teliospores are slightly larger (6.5–10)×(7–11) µm after one month. The second type of teliospore is brown, thick-walled, ovoidal (9–11)×(14–15) µm or subglobose (7.5–17)×(8–18.5) µm with a large globular inclusion and is produced in groups of up to 12.

Germination of the teliospores can be achieved as follows: the teliospores are harvested from dikaryotic cultures grown on corn meal agar for 17 days and soaked in distilled water for 20 weeks before transferring to 2% water agar. The teliospores germinate after 3 days on 2% agar. The morphology of metabasidia is variable and, most commonly, they are pyriform with a large base attached to the teliospore (Fig. 318B). The base of the metabasidia is subglobose (5–7)×(7–9) µm or, occasionally, ovoidal-elongate (3.5–8 µm), and the apex is globose (1.5–3.5 µm) to subglobose (2–4)×(2.5–5) µm. In some metabasidia, the apex is two-celled, with the terminal cells as large as, or slightly larger than, the basal cell. Sporidia are 1–4 in number and develop terminally on the metabasidium. Lateral sporidia may be found only rarely. Endospores, in numbers of up to 10, may develop within the mycelium. Conjugation does not occur when any of the mating types are mixed with the other species of *Cystofilobasidium.*

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	+
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	w
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

D-Saccharate	–	Thiamine-free	–
Splitting of arbutin	+	Biotin-free	–
50% Glucose (w/w)–yeast extract agar	–	Growth at 30°C	+/w
Starch formation	+	Growth at 33°C	–
Urease	+		

Co-Q: 8 (Yamada and Kondo 1973).
Mol% G+C: 66.3 (HPLC: Hamamoto et al. 1988b).

Origins of the strains studied: Marine waters of the Antarctic, Southern Indian, and Southern Pacific Oceans (14); human source (1) (Fell et al. 1973); air in Tokyo (1) (Okunuki 1931); diseased pears in China as *Rhodotorula sinensis* (1) (Lee 1974).

Complementary mating types: CBS 6350 (mating type *A2*) and CBS 6352 (mating type *A3*), both from marine sources.

Type strain: CBS 323 (mating type *A1*), the type strain of *Torula infirmominiata.*

Comments: *C. infirmominiatum* is the only known species in the family Filobasidiaceae that produces pyriform metabasidia. It is unique in two other ways: it is the only known species in the family that has a unifactorial incompatibility system with three alleles, and it is the only known species in the genus *Cystofilobasidium* that lacks primary sporidia.

In addition to marine water, *C. infirmominiatum* has been isolated from frozen vegetables, raw meat, air, diseased pears, and fecal samples of a man with symptoms of tropical sprue. There is no evidence that the yeast is pathogenic for humans.

80.4. *Cystofilobasidium lari-marini* (Saëz & T.L. Nguyen) Fell & Statzell-Tallman (1992)

Synonym:
Leucosporidium lari-marini Saëz & T.L. Nguyen (1989)

Growth in 2% malt extract broth: Moderate growth with ring and fragile pellicles after one month.

Growth on 2% malt extract agar: After 3 days at 12°C, the cells are ellipsoid or ovoid, (2–5)×(5–12) µm, and single or in pairs (Fig. 320A). Reproduction is by polar budding.

Growth in 2% glucose–peptone agar (Sabouraud agar): Colonies are white, and semi-glossy. Submerged mycelium and pseudomycelium are produced after 2 to 3 weeks. Spheroidal, ovoidal, or pyriform teliospores, (7.5–10)×(10–20) µm, are produced singly or in groups of 2 to 3 on mycelium at either terminal or intercalary position. Teliospores are spheroidal (10.6–13.3 µm) with apical swelling (4–8 µm); 4–8 sessile, oblong basidiospores are produced on the apical portion of the metabasidia (Fig. 320B,C). Basidiospores germinate by budding.

Teliospores germinate one week after soaking in water, heating for one hour at 57°C and incubating at 25°C, followed by plating on corn meal agar (Fell and Statzell-Tallman 1992).

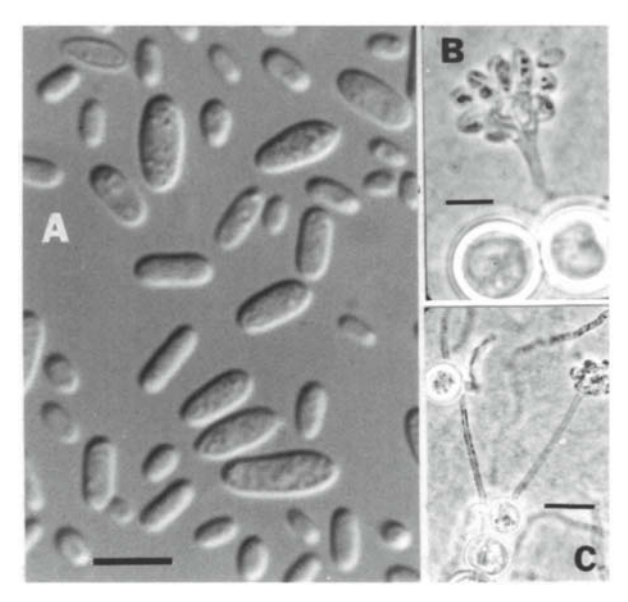


Fig. 320. *C. lari-marini.* (A) Yeast cells grown on malt extract agar. Bar = 10 µm. (B) Basidium with relatively short metabasidium. Bar = 5 µm. (C) Basidium with long metabasidium. Bar = 13 µm. (Fell and Statzell-Tallman 1992).

Fermentation:

Glucose	w	Lactose	–
Galactose	–	Raffinose	w/–
Sucrose	w/–	Trehalose	ws
Maltose	w/–		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+/w
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	+
Lactose	+/w	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+/w	Inositol	+
D-Ribose	+/w	Hexadecane	–
L-Rhamnose	+/w	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

50% Glucose	+	Cycloheximide 0.05%	–
Starch formation	+/w	Growth at 37°C	+
Urease	+		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: CBS 7420 was isolated from the mouth cavity of a dead sea gull.

Type strain: CBS 7420.

Comments: *Cystofilobasidium lari-marini* was previously classified in the genus *Leucosporidium* because it produces white colonies with teliospores (Saëz and Nguyen 1989). Saëz and Nguyen (1989) did not describe the germination of teliospores or the formation of metabasidia. Studies on the nucleotide sequence of a variable region of the large subunit rRNA, however, indicated the close relationship of *L. lari-marini* with *Cystofilobasidium* rather than with *Leucosporidium* (Fell and Kurtzman 1990). In a subsequent study, Fell and Statzell-Tallman (1992) confirmed the relationship of *L. lari-marini* to *Cystofilobasidium* by demonstrating *C. capitatum*-like metabasidium formation in *L. lari-marini.*

Comments on the genus

Oberwinkler and Bandoni (1983) established the genus *Cystofilobasidium* to accommodate carotenoid-pigmented yeasts which produce hyphae with primitive dolipores and which form teliospores that germinate to produce holometabasidia with terminal basidiospores. They included two species in the genus: *C. bisporidii* and *C. capitatum*; both had been classified in the genus *Rhodosporidium* by Fell et al. (1973). Hamamoto and coworkers (1988b) added another species, *C. infirmominiatum* to the genus on the basis of DNA–DNA homology with the other *Cystofilobasidium* spp., and because of certain biochemical characteristics of the species. *C. infirmominiatum* had also been classified in the genus *Rhodosporidium* by Fell et al. (1973). Fell (1974) was aware of the major differences between these three species and the other *Rhodosporidium* spp., in the context of metabasidium morphology and biochemical characteristics. Unlike the typical species of *Rhodosporidium,* which produce phragmobasidia and mycelium with simple septa, *Cystofilobasidium* species produce holometabasidia and mycelium with dolipores (without parenthesomes). In contrast to *Rhodosporidium* species, *Cystofilobasidium* assimilates inositol and produces starch-like compounds. The two genera can also be distinguished on the basis of their whole-cell sugar composition and coenzyme Q systems. Cells of *Cystofilobasidium* species contain xylose, while it is lacking in *Rhodosporidium* (Weijman et al. 1982, Weijman and Rodrigues de Miranda 1988, von Arx and Weijman 1979). *Cystofilobasidium* contains ubiquinone Q-8 (Yamada and Kondo 1973, Sugiyama et al. 1985), in contrast to *Rhodosporidium* which forms Q-9 or Q-10. Yamada and Kawasaki (1989a) compared partial sequences of 18S and 26S rRNAs from Q-8-containing species of teliospore-forming yeasts, and reported that the three *Cystofilobasidium* spp. cluster in the same group and are distinguishable from the other groups.

Species of *Cystofilobasidium* share morphological similarities with the other two genera of the Filobasidiaceae, *Filobasidiella* and *Filobasidium,* by producing filobasidiaceous metabasidia; however, the presence of teliospores clearly separates the genus from *Filobasidium* and *Filobasidiella.* Other features that distinguish *Cystofilobasidium* from the other two genera of the Filobasidiaceae include its formation of carotenoid pigment in the yeast phase, formation of endospores in the mycelium, and the non-deciduous nature of primary basidiospores. Unlike *Cystofilobasidium,* species belonging to *Filobasidium* and the Filobasidiaceae have coenzyme Q-10 system. The anamorphs of *Cystofilobasidium* spp. belong to the genus *Rhodotorula* (Fell and Statzell-Tallman 1984) or the genus *Cryptococcus.* Three of the four species produce bright orange, salmon-pink to cinnamon-colored, glistening colonies. *C. lari-marini* can be distinguished from the other three species by the lack of carotenoid pigments. *C. bisporidii* can be distinguished from the other two species with carotenoid pigments by its ability to assimilate melibiose. *C. infirmominiatum* is separated from *C. capitatum* by splitting of arbutin, the inability to assimilate L-sorbose, and the formation of pyriform metabasidia.

Study of the life cycle of *Cystofilobasidium* is impaired because of the low frequency of mating and the difficulty in germinating teliospores. The teliospores may germinate after growing on malt or corn meal agar for three weeks, followed by 8–11 weeks of soaking in distilled water in most species (Fell et al. 1973).

81. *Erythrobasidium* Hamamoto, Sugiyama & Komagata

J. Sugiyama and M. Hamamoto

Diagnosis of the genus

Asexual reproduction is by multilateral budding. Cells are ovoidal to long ovoidal and pseudomycelium is absent. The colony color on solid media is orange-red.

True mycelia are produced from single cells without mating. Clamp connections are present or absent. Septal pores are simple. Teliospores are not formed. Unicellular basidia (holobasidia) arise by the development of the lateral protrusions on the hyphae. The sessile basidiospores are produced terminally on the holobasidia; these are non-apiculate and are not forcibly discharged.

Fermentation is absent. The urease test is positive. The extracellular DNase test is positive (weak). The diazonium blue B color test is positive. Xylose is absent in the cells. The major ubiquinone system is Q-10(H_2). D-Glucuronate and nitrate are assimilated; *myo*-inositol is not assimilated. Starch-like compounds are not produced.

Type species

Erythrobasidium hasegawianum Hamamoto, Sugiyama & Komagata

Species accepted

1. *Erythrobasidium hasegawianum* Hamamoto, Sugiyama & Komagata (1991)

Systematic discussion of the species

81.1. *Erythrobasidium hasegawianum* Hamamoto, Sugiyama & Komagata (1991)

Anamorph: *Rhodotorula hasegawae* Y. Yamada & Komagata
Synonyms:
Erythrobasidium hasegawae (Y. Yamada & Komagata) Hamamoto, Sugiyama & Komagata (1988c), incorrect combination, Art. 59, ICBN
Rhodotorula hasegawae Y. Yamada & Komagata (1983)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to long ovoidal, (3.8–5.8)×(6.3–10.5) μm, and single or in pairs. A sediment and a ring are present.

Growth on 5% malt agar: After 3 days at 25°C, the cells are of the same form and almost the same dimensions, (3.3–5.6)×(6.3–12.0) μm, as observed in malt extract. The streak culture is smooth, glistening and pink to reddish-pink. After one month at room temperature, the culture is orange-red.

Dalmau plate culture on corn meal agar: After 10 days at 20°C, there is true mycelium. The mycelium is initially uninucleate; during the process of clamp formation, the clamps are completed but are not always present at each cross wall (Fig. 321A). Hyphae are dikaryotic when clamp connections are formed, and uninucleate when they are not formed (Fig. 321B). After 50–60 days, protrusions are produced laterally on the hyphae and develop into uninucleate basidia (Fig. 321C). These are obovate, 10–18 μm long, 1–2 μm wide near the base, and 3–5 μm wide at the apex, single, and one-celled. They appear to be diploid as their nuclei are twice as large as those of the yeast cells. The basidium produces two to four terminally sessile basidiospores on its apices.

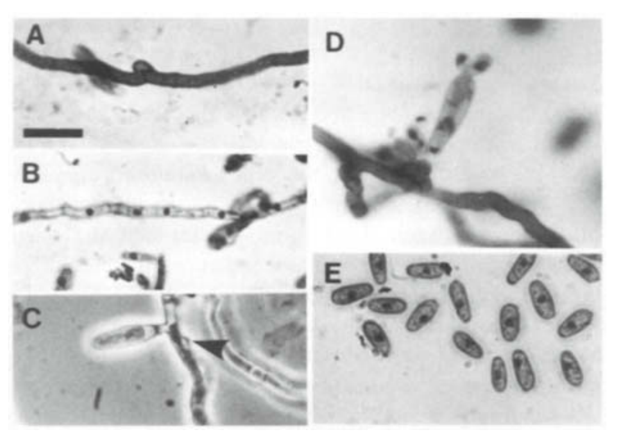


Fig. 321. *E. hasegawianum*, IFO 1058. (A) Hyphae with clamp connections, after 10 days on corn meal agar at 20°C. (B) Uninucleate mycelium stained by the Giemsa method. (C) Basidium after 50–60 days on corn meal agar at 20°C. Arrow indicates a protrusion. (D) Germinated basidium with formation of uninucleate basidiospores, stained by the Giemsa method. (E) Haploid yeasts stained by the Giemsa method. Bar = 10 μm.

These basidiospores apparently are haploid (Fig. 321D, E). Subsequently, basidia repeat the budding.

Life cycle: A dikaryotic mycelium with clamp connections or a uninucleate mycelium without clamps develops from a single cell without mating (Fig. 322). The mycelium produces protrusions laterally. The protrusion develops into uninucleate obovate holobasidia. Karyogamy may take place in the holobasidium. Meiosis may occur during germination, and the resulting sessile haploid basidiospores are terminal on the holobasidium. The basidiospores propagate by budding, thus returning to the yeast phase.

Fermentation: absent.

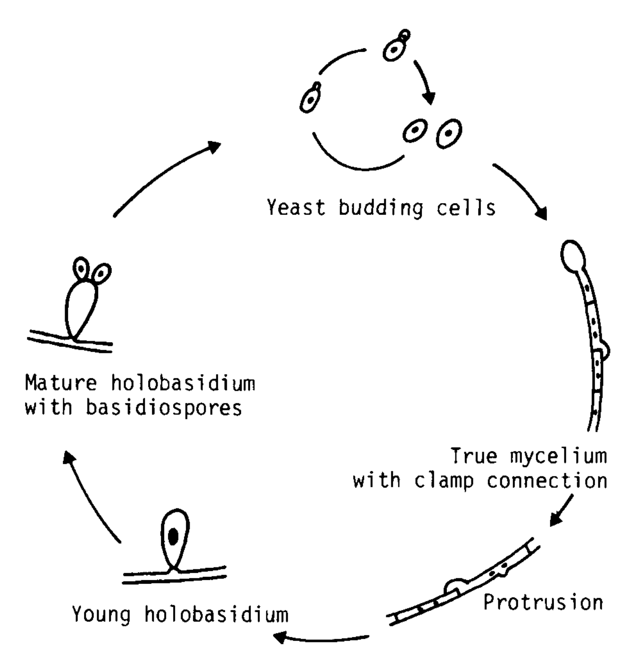


Fig. 322. Life cycle of *E. hasegawianum.*

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	w	Erythritol	–
Cellobiose	s	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	s	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
Arbutin splitting	+	Extracellular DNase	+/w
Starch formation	–	Growth at 37°C	–

Co-Q: 10(H_2) (Yamada et al. 1973c).

Mol% G+C: 55.7 (HPLC: Hamamoto et al. 1986b); 54.8 (T_m: Hamamoto et al. 1986b); 50.0 (T_m: Nakase and Komagata 1971c), all values were determined from IFO 1058.

Xylose in the cells: Absent (Sugiyama et al. 1985).

Septal pore structure: Simple (Suh et al. 1993a).

Origin of the strain studied: An old culture of beer yeast, Philadelphia, U.S.A. (Robbins and Ma 1944).

Type strain: IFO 1058 (ATCC 9536, CBS 8253, IAM 12911, JCM 1545, YK 124), derived from the holotype IAM F-0146; isolated from an old culture of beer yeast, Philadelphia, U.S.A.; received from IFO.

Comments on the genus

Hamamoto et al. (1988c) discovered the teleomorphic state of *Rhodotorula hasegawae,* which is characterized by unicellular basidia and the lack of teliospores. They proposed the new genus *Erythrobasidium* for the taxon and tentatively placed it in the Filobasidiaceae. Later Hamamoto et al. (1991) replaced the species name *E. hasegawae* (Yamada & Komagata) Hamamoto et al. (1988c) by a new name *E. hasegawianum* Hamamoto et al. (1991) because of an incorrect combination for the teleomorph.

Only one strain (IFO 1058) is known for this species. The strain, isolated from an old culture of beer yeast, was first identified as *Rhodotorula aurantiaca* (Robbins and Ma 1944) and subsequently reidentified as *R. lactosa* (Hasegawa 1965) on the basis of assimilation of lactose and the requirement for *p*-aminobenzoic acid. Later Yamada and Komagata (1983) pointed out that the strain was distinguished from both *R. aurantiaca* and *R. lactosa* by DNA base composition, Co-Q system and the electrophoretic patterns of enzymes. Furthermore, it did not assimilate lactose, contrary to the description of Hasegawa (1965). On the basis of these data, Yamada and Komagata (1983) proposed the new species *R. hasegawae.*

Suh et al. (1993a) have clarified the ultrastructural features of *E. hasegawianum.* They showed the cell wall to be multilayered, conidiogenesis to be enteroblastic and that the septal structure is simple with no septal swelling or parenthesomes. Such features strongly suggest that *Erythrobasidium* has affinity with the teliospore-forming yeasts rather than with Filobasidiaceous taxa. 18S rRNA gene sequence comparisons among the basidiomycetous yeasts supports conclusions from the ultrastructural data (Sugiyama and Suh 1993, Suh and Sugiyama 1993b). Subsequently, Suh and Sugiyama (1994) suggested a phylogenetically close relationship between *E. hasegawianum* and *Rhodosporidium dacryoideum* using neighbor-joining analysis of 18S rRNA gene sequences. For further discussion, see Suh and Sugiyama (1994).

82. *Filobasidiella* Kwon-Chung

K.J. Kwon-Chung

Diagnosis of the genus

Asexual reproduction is by budding of globose, ovoidal, or apiculate cells. Pseudo- or true mycelium is lacking in haploid cultures. Growth on solid media is white or cream-colored, and commonly mucoid. Visible carotenoid pigments are lacking. Cells are encapsulated by polysaccharide, and the degree of capsulation varies with strains as well as environmental factors.

Sexual reproduction occurs following conjugation between α and *a* cells. Conjugated cells produce dikaryotic hyphae with clamp connections. The cross walls of the hyphae contain a typical dolipore, but lack parenthesomes (Kwon-Chung and Popkin 1976). Basidiocarps are absent. A slender holobasidium with an abruptly expanded apex arises laterally or terminally from the dikaryotic hyphae. Karyogamy takes place in the metabasidium, and the zygote nucleus undergoes meiosis in the apical area. Repeated mitosis follows meiosis, and uninucleate basidiospores bud basipetally from four spots on the apex of the metabasidium to form four chains of basidiospores.

Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated, but nitrate is not assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is formed. Xylose is present in whole-cell hydrolyzates.

Type species

Filobasidiella neoformans Kwon-Chung

Species accepted

1. *Filobasidiella neoformans* Kwon-Chung (1975)
   a. *Filobasidiella neoformans* Kwon-Chung var. *neoformans* (1982)
   b. *Filobasidiella neoformans* var. *bacillispora* Kwon-Chung (1982)

Key to the varieties

1. a Basidiospores spheroidal, oblong, or cylindroidal and finely roughened; D-proline is not assimilated; reaction on canavanine-glycine-bromthymol blue (CGB) agar is negative after two to five days .... *F. neoformans* var. *neoformans*: p. 656
   b Basidiospores smooth, bacillary and with or without curvature; D-proline is assimilated; CGB agar becomes blue within two to five days .... *F. neoformans* var. *bacillispora*: p. 657

Systematic discussion of the species

82.1. *Filobasidiella neoformans* Kwon-Chung (1975)

This species has two varieties:

Filobasidiella neoformans Kwon-Chung var. *neoformans* (Kwon-Chung et al. 1982a)

Anamorph: *Cryptococcus neoformans* (Sanfelice) Vuillemin

Synonyms:

Saccharomyces neoformans Sanfelice (1895a,b)
Cryptococcus neoformans (Sanfelice) Vuillemin (1901)
Debaryomyces neoformans (Sanfelice) Redaelli, Ciferri & Giordano (1937)
Saccharomyces lithogenes Sanfelice (1895b)
Cryptococcus lithogenes (Sanfelice) Vuillemin (1901)
Blastomyces lithogenes (Sanfelice) Sasakawa (1922)
Torulopsis lithogenes (Sanfelice) de Almeida (1933)
Cryptococcus hominis Vuillemin (1901) pro parte
Saccharomyces hominis Costantin (1901)
Atelosaccharomyces hominis (Vuillemin) Verdun (1912)
Torulopsis hominis (Vuillemin) Redaelli (1931)
Debaryomyces hominis (Vuillemin) Todd & Herrmann (1936)
Cryptococcus costantini Froilano de Mello & Gonzaga Fernandes (1918)
Torulopsis costantini (Froilano de Mello & Gonzaga Fernandes) de Almeida (1933)
Saccharomyces plimmeri Costantin (1901)
Torula plimmeri (Costantin) Weis (1902)
Cryptococcus plimmeri (Costantin) Neveu-Lemaire (1912)
Torulopsis plimmeri (Costantin) de Almeida (1933)
Torula klein Weis (1902)
Cryptococcus kleini (Weis) Cohn (apud Guéguen 1904)
Atelosaccharomyces busse-buschki de Beurmann & Gougerot (1909)
Saccharomyces blanchardi Guiart (1910)
Atelosaccharomyces breweri Verdun (1912)
Cryptococcus breweri (Verdun) Castellani & Chalmers (1913)
Saccharomyces breweri (Verdun) Neveu-Lemaire (1921)
Torulopsis breweri (Verdun) de Almeida (1933)
Torula histolytica Stoddard & Cutler (1916) pro parte
Torulopsis histolytica (Stoddard & Cutler) Castellani & Jacono (1933)
Cryptococcus cerebriloculosis Freeman & Weidman (1923)
Torula nasalis Harrison (1928)
Cryptococcus nasalis (Harrison) Dodge (1935)
Cryptococcus hondurianus Castellani (Castellani and Jacono 1933)
Cryptococcus hominis Vuillemin var. *hondurianus* Castellani (Castellani and Jacono 1933)
Torulopsis hominis (Vuillemin) Redaelli var. *honduriana* Castellani (Castellani and Jacono 1933)
Cryptococcus psicrophilicus Niño (1934)
Torulopsis neoformans (Sanfelice) Redaelli var. *sheppei* Giordano (1935)
Cryptococcus meningitidis Dodge (1935)

***Filobasidiella neoformans* var. *bacillispora* Kwon-Chung (Kwon-Chung et al. 1982a)**

Anamorph: *Cryptococcus neoformans* (Sanfelice) Vuillemin var. *gattii* Vanbreuseghem & Takashio

Synonyms:

Filobasidiella bacillispora Kwon-Chung (1976a)

Cryptococcus bacillisporus Kwon-Chung & Bennett (Kwon-Chung et al. 1978)

Cryptococcus neoformans (Sanfelice) Vuillemin var. *gattii* Vanbreuseghem & Takashio (1970)

Growth in 2% malt extract: After 3 days at 25°C, haploid cells are mostly globose or ovoidal, occurring singly or with buds (Fig. 323C). The cell size ranges from 3–8 µm, but is mostly 3–5 µm in diameter. A slight sediment is present, and a thin ring may be present as well. The sediment increases as the incubation continues.

Growth on 2% malt agar: After 3–5 days at 25°C, haploid cells are globose to ovoidal, measuring 2.5–10 µm in diameter. The colony is white to cream-colored with a smooth, mucoid texture; the margin is entire. In streak culture, the highly mucoid growth runs to the bottom of the slant within one week.

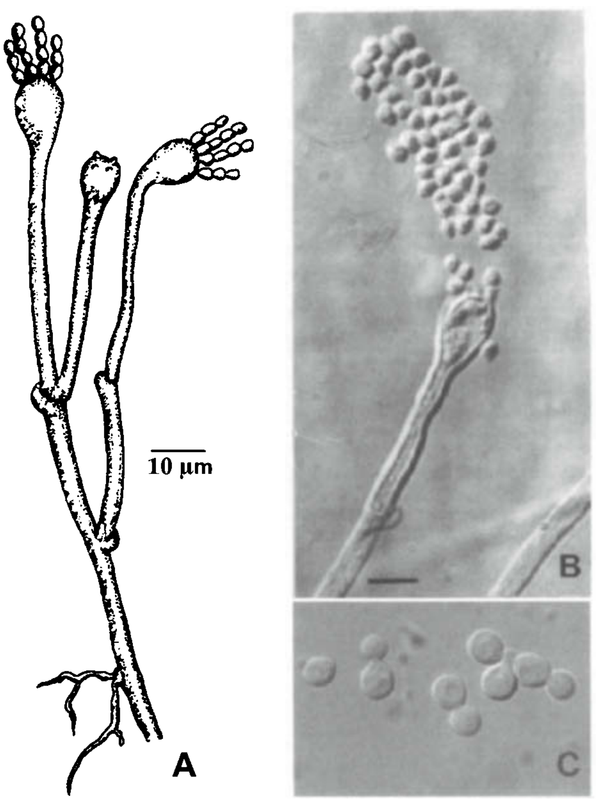


Fig. 323. *F. neoformans* var. *neoformans*. (A) Basidial structure with "hausteriod" hyphae. (B) Basidiospores are released from the basidium. Bar = 10 µm. (C) Yeast cells grown on malt extract agar.

Dalmau plate culture on corn meal agar: Pseudomycelium or mycelium is absent in haploid cells.

Growth on agar medium containing phenolic compounds: Haploid colonies on media containing substances such as extract of niger seed (*Guizotia abyssinica*) (Staib 1962, Shields and Ajello 1966), caffeic acid (Pulverer and Korth 1971, Hopfer and Blank 1975, Hopfer and Gröschel 1975, Healy et al. 1977, Fleming et al. 1977), chlorogenic acid (Shaw and Kapica 1972) or, L-DOPA (Chaskes and Tyndall 1975) become brown or dark brown to black usually within one to five days, depending on the medium and the isolates. Some strains may require eight days to produce the characteristic pigment on niger seed agar.

Growth on canavanine-glycine-bromthymol blue (CGB) agar: There is no growth, and the color of the medium does not change after two to five days at 25°C.

Life cycle: The life cycle of *F. neoformans* is illustrated in Fig. 324. The majority of isolates are self-sterile and produce basidiospores upon mating with isolates of the opposite mating type. Heterothallism is controlled by a one-locus, two-allele (α and a) system. Cells of α and a mating types conjugate when they are mixed on malt extract agar, V8 juice agar, hay infusion agar, or sucrose yeast extract agar at 25–37°C. V8 juice with 4% agar and hay infusion agar have been the two media most effective for formation of the metabasidia.

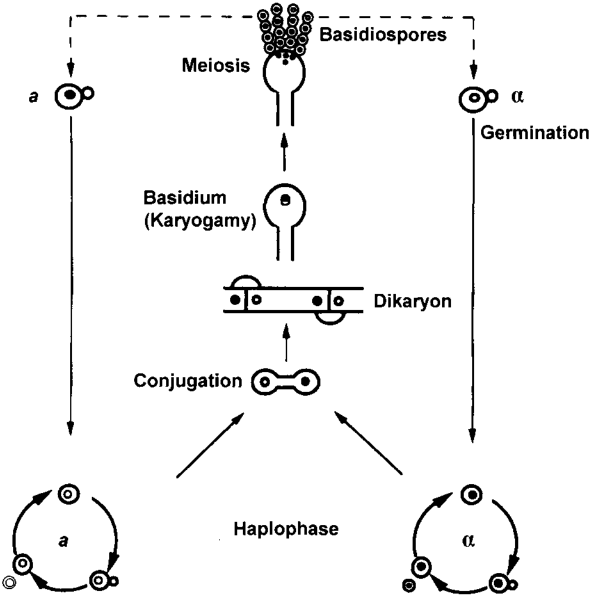


Fig. 324. Life cycle of *F. neoformans*.

Dikaryotic hyphae with clamp connections develop within three to five days from the conjugants. After one week, mycelial growth is most evident around the margin of the colony. Hyphae are thin (average 3 µm wide) and hyaline, with a clamp connection at each septum. Regular hyphal branching occurs at clamp connections, and also irregular, thin (1–1.5 µm wide) branches termed "haustorioid" branches originate from clamps. Blastospores bud out from the side or the tip of the hyphae; these are indistinguishable from haploid yeast cells in their size and shape. Mature basidia arise within one to two weeks, singly or in a group, laterally

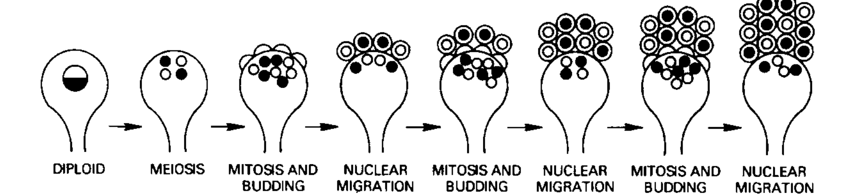


Fig. 325. Nuclear division and the formation of basipetal basidiospore chains in *F. neoformans* (Kwon-Chung and Fell 1984).

or terminally on the hyphae. Basidia are nonseptate, slender, and uniformly wide in the basal area, but expand abruptly to form oblong to clavate apices (4–10)×(6–13) µm (Fig. 323A,B).

The apical enlargement is often at an angle to the main axis of the basidium. Diploidization occurs in the basidium, and meiosis takes place near the apex of the basidium. Basidiospores are ovoidal, ellipsoidal, cylindroidal, pyriform to globose (1.8–3×2–5 µm in diameter), with finely roughened walls and with or without truncate bases. They are produced by basipetal budding directly from four spots on the basidial apex. The chain of basidiospores may contain more than 20 spores per chain. The basidiospore chains collapse into spore balls under humid conditions. Some basidia produce fewer than four initial spores, and these spores are usually twice the size of typical spores. Rarely, more than four initial basidiospores are produced, and extensive chain formation has not been observed in such basidia. Initially, spores are dry and a capsule is not visible until the spores enlarge to twice the original size and undergo profuse budding. Budding of basidiospores results in mucoid colonies of yeast cells representing *α*, *a*, or infrequently *αa* (self-fertile) mating types.

Cytological (Kwon-Chung 1976b) and genetic (Kwon-Chung 1980) studies indicate that the four small nuclei of the first meiotic product remain in the apical part of the basidium and each nucleus divides mitotically. Daughter nuclei migrate into basidiospores, while parent nuclei divide repeatedly during basipetal budding to form uninucleate basidiospores in chains. The spore chains show random distribution of nuclei and, as a result, the spores from a single chain are not genetically identical (Fig. 325).

Monokaryotic fruiting: Shadomy (1970) demonstrated hyphal formation in 11 single cultures (Lurie and Shadomy 1971), and three of the hyphae-forming isolates completed basidial formation without apparent mating (Erke 1976). The isolates were all from human cryptococcosis cases. The isolates first grew in yeast form and produced hyphae with clamp connections either from single yeast cells or from a pair of yeast cells connected by a clamp connection. Hyphal cells were monokaryotic but dikaryons were observed in terminal cells in some strains. In some strains, however, dikaryons were not restricted to terminal cells. The morphological aspects of the basidium and basidiospores produced in self-fertile strains were the same as in the mated cultures except that in the former, hyphae showed incomplete clamp connections (clamps not fused to the adjoining cells). Colonies developed from single basidiospore cultures obtained from such fruitings were capable of repeating the whole life cycle, but the yeast cells isolated from such colonies became markedly weaker in sporulation in subsequent generations. Erke (1976) suggested that these strains are primarily homothallic; and others designated these strains as self-fertile, though principally heterothallic (Kwon-Chung 1977a). The genetic basis of the self-fertility in these strains was hypothesized to be a diploid state, containing both *α* and *a* alleles. Recently, it was found that this so-called self-fertility is a monokaryotic fruiting primarily occurring in the *α* isolates. Monokaryotic fruiting has been observed in many genera of the Homobasidiomycetes (Stahl and Esser 1976).

Wickes et al. (1996) observed that a majority of *α* isolates tested can produce monokaryotic fruiting on a medium deprived of nitrogen such as Yeast Nitrogen Base without ammonium sulfate. The association of the *α* mating type with "self-fertility" had been noted twenty years ago (Kwon-Chung 1977a), when it was found that all the self-fertile cultures eventually behave as *α* type when the self-fertility becomes suppressed during the prolonged maintenance on rich media. This phenomenon, however, was thought to be due to the loss of the *a* factor.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	v
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	w
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+/w	Ribitol	v
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+/w	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	v	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	+/w	Citrate	v
D-Arabinose	+	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

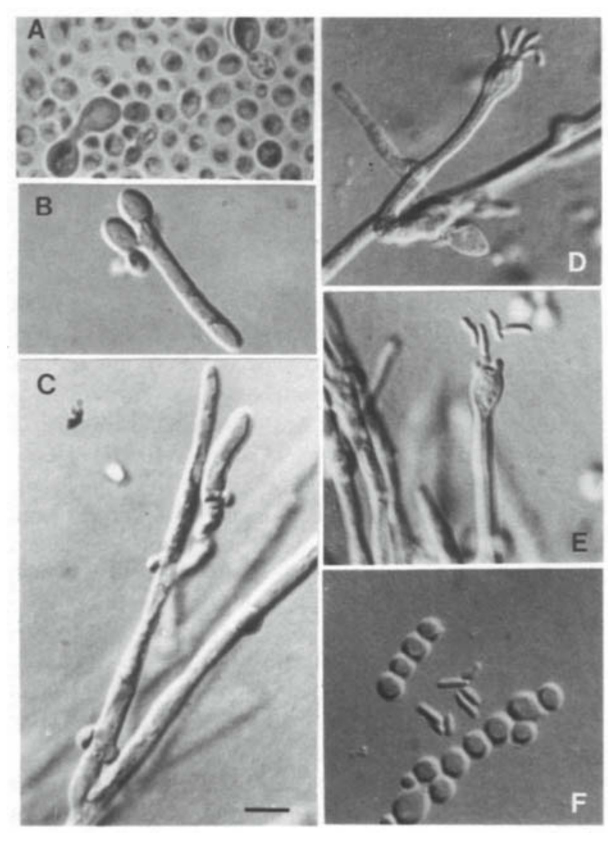


Fig. 326. *F. neoformans* var. *bacillispora*. Bar = 10 µm. (A) Conjugating cells. (B) Hyphal formation by a pair of conjugated cells. (C) Hyphae with clamp connections. (D) The first set of four basidiospores are produced at the apex of a basidium. (E) Release of the first set of basidiospores and production of the second set of basidiospores. (F) The contrast between yeast cells and basidiospores (Kwon-Chung 1976a).

Additional assimilation tests and other growth characteristics:

Splitting of arbutin	+	Urease	+
L-Malate	v	Growth on 50% w/w glucose–yeast extract agar	–
Ascorbic acid	+		
Thiamine-free	–	Cycloheximide 12 µm/ml	–
D-Saccharate	v	Growth at 37°C	+
Starch formation	+	Growth at 40°C	v

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G + C: 53.2–56.7, three strains (T_m: Aulakh et al. 1981).

Supplementary description of *F. neoformans* var. *bacillispora*: This variety can be readily differentiated from the var. *neoformans* by morphological and biochemical characteristics. In contrast to *F. neoformans* var. *neoformans*, *F. neoformans* var. *bacillispora* produces smooth and bacillary-shaped basidiospores measuring (1–1.8) × (3–8) µm, but produces no haustorioid hyphae. The anamorph of *F. neoformans* var. *bacillispora*, *Cryptococcus neoformans* var. *gattii*, tends to produce more oval- and lemon-shaped, (3–5) × (3–7) µm, yeast cells rather than globose cells. The growth rate of the two varieties is the same at 25°C, but at 37°C, var. *bacillispora* grows slower than var. *neoformans*. Unlike *F. neoformans* var. *neoformans*, the var. *bacillispora* can utilize D-proline as a sole source of nitrogen and changes the color of CGB medium to blue-green within two to five days. The life cycle of var. *bacillispora* resembles that of *F. neoformans* except that haustorioid hyphae have not been observed (Fig. 326).

F. neoformans var. *bacillispora* has not been isolated from pigeon droppings or soil and does not share an ecological niche with the *neoformans* variety. The natural habitat of this variety is reported to be the Red River gum tree, *Eucalyptus camaldulensis* (Ellis and Pfeiffer 1990).

Mol% G + C of the variety *bacillispora*: 56.3–57.2, three strains (T_m: Aulakh et al. 1981).

Origin of the strains belonging to the variety *neoformans*: CBS 132 (type strain of *Cryptococcus neoformans*) from peach juice; CBS 6885 (α type) from a human bone lesion; CBS 6886 (a type) and NIH 433 isolated from pigeon excreta in Denmark; CBS 6900 and CBS 6901, single basidiospore progenies obtained from CBS 6885 × NIH 433. The natural habitat is pigeon and other avian excreta and soil.

Complementary mating types and living reference strains: CBS 6885 (α type) and CBS 6886 (a type).

Holotypes: BPI 71843, a slide mount of basidial structures prepared from the cross CBS 6885 (α) × CBS 6886 (a), was deposited in the herbarium of the National Fungus Collection, Beltsville, MD. The holotype of the type culture of *Cryptococcus neoformans* var. *neoformans* (BPI 72042) has also been deposited at the National Fungus Collection.

Origin of the strains belonging to the variety *bacillispora*: Eleven strains from human cases of cryptococcosis. CBS 6992 (serotype B) and CBS 6993 (serotype C) were originally received from Dr. Evans.

Complementary mating types and living reference strains: CBS 6956 (α mating type, serotype B, from sputum) and CBS 6955 (a mating type, serotype C, from spinal fluid).

Holotypes: A slide mount (BPI 71855) of basidial structures prepared from the cross CBS 6992 × CBS 6993 has been deposited in the herbarium, National Fungus Collection, Beltsville, MD. The holotype of *Cryptococcus neoformans* var. *gattii*, and the anamorph or *F. neoformans* var. *bacillispora*, are preserved at the Laboratory for Mycology of the Institute of Tropical Medicine, Antwerp, Belgium (De Vroey and Gattii 1989).

Comments on the genus

The disease caused by *Cryptococcus neoformans*, the haploid state of *F. neoformans*, was first reported by Busse in 1894. Busse was a pathologist who observed the round to oval "corpuscles" in a tumor of the tibia of a woman (Busse 1894) who was diagnosed with a soft sarcoma. Busse isolated a culture from the lesion, and one of his colleagues thought that it was a pathogenic species of blastomycetes (*fide* Sanfelice 1895a). Buschke, a physician who took care of the patient, thought that the etiologic agent was a coccidia (Buschke 1895).

Soon after Busse's report, Sanfelice isolated an encapsulated yeastlike fungus from peach juice and named it *Saccharomyces neoformans* (Sanfelice 1894). In 1895, Sanfelice proved the pathogenicity of *S. neoformans* by inoculating the yeast into experimental animals (Sanfelice 1895a). He recognized the similarity between *S. neoformans* and Busse's isolate. In the same year, he isolated a "*Saccharomyces*" which was thought to be the same fungus as *S. neoformans* from the lymphatic glands of an ox which had died from a primary liver infection (Sanfelice 1895b).

In 1901, Vuillemin transferred the fungus to the genus *Cryptococcus* as *C. hominis,* because he did not find ascospores that characterize the genus *Saccharomyces* (Vuillemin 1901). *Cryptococcus neoformans* had been regarded as a homogeneous species except for the existence of four serotypic variations, A, B, C, and D (Evans 1949, 1950, Wilson et al. 1968) among the isolates. Since the discovery of two morphologically different sexual states among the isolates of *C. neoformans,* it became clear that the isolates of serotypes B and C are fundamentally different from the isolates of serotypes A and D (Bennett and Kwon-Chung 1989). Initially, *C. neoformans* was considered to contain two different teleomorphic species: *F. neoformans* (Kwon-Chung 1975) and *F. bacillispora* (Kwon-Chung 1976a). The *F. neoformans* state was produced by the cross between two mating types of serotype A or D isolates, whereas the *F. bacillispora* state was observed when crosses were made among serotype B or C isolates. Intercrossing between A, D and B, C isolates usually produces basidia with two types of spores: oblong to lemon-shaped spores of *F. neoformans* type, and bacilliform spores of *F. bacillispora* type. In some crosses, however, the spores were predominantly of *F. bacillispora* type (Rogers et al. 1980). The spores produced by the intercrossings between the type cultures of the two species and many other pairs were sterile; however, CBS 6991 (*a* type, serotype D) produced viable basidiospores (30%) when crossed with CBS 6956 (α type, serotype B). The viable basidiospores produced a 1:1 ratio of α and *a* mating types, but all were serotype D. All the α type F_1 isolates assimilated L-malate, while *a* type did not. Since 70% of the basidiospores were nonviable, it was not possible to analyze whether the viable progeny were recombinants or whether the D factor was a dominant cytoplasmic factor. Further genetic analyses between the two species of *Filobasidiella* indicated that they can be more appropriately classified as two varieties of one species. Aulakh et al. (1981) showed 55–63% relatedness between the DNAs of *F. neoformans* and *F. bacillispora.* Furthermore, the type strain of *C. neoformans* var. *gattii* Vanbreuseghem and Takashio (1970) mated with *F. neoformans* and produced basidiospores. The type culture of *C. neoformans* var. *gattii* was physiologically and morphologically indistinguishable from the anamorph of the type strain of *F. bacillispora.* On the basis of these results, *F. bacillispora* was reclassified as *F. neoformans* var. *bacillispora* (Kwon-Chung et al. 1982a).

Cryptococcus neoformans var. *neoformans,* the haploid state of *F. neoformans* var. *neoformans,* and *C. neoformans* var. *gattii,* the haploid state of *F. neoformans* var. *bacillispora,* show considerable differences in their electrophoretic karyotype. Wickes et al. (1994) were able to separate 13 chromosome-sized DNA bands in the reference strains of *F. neoformans* var. *neoformans* and 13–14 bands in the reference strains of *F. neoformans* var. *bacillispora,* but the smallest band of *F. neoformans* var. *neoformans* was approximately 250 kb larger than that of the var. *bacillispora.* The anamorphs of the two varieties also show differences in ecology and epidemiology. Pigeon droppings and, less frequently, other bird droppings have been known as the major environmental source of *C. neoformans* var. *neoformans* (Emmons 1955). Pigeons seem to be a carrier of *C. neoformans* var. *neoformans,* since pigeons caged in different parts of the world contain the fungus in their crops. Our test with 11 isolates obtained by Vanbreuseghem from pigeon crops (caged pigeons) showed all to be serotype A. Isolates of serotype D were frequently found in pigeon droppings collected in northern Europe (Bennett et al. 1977). Although infrequent, *C. neoformans* var. *neoformans* has been isolated from rotting vegetables, fruits and fruit juices, wood, dairy products, and soil contaminated with pigeon droppings.

The ecological niche of *F. neoformans* var. *neoformans* is not known. It is suspected that the natural reservoir of *F. neoformans* var. *neoformans* is different from that of *C. neoformans* var. *neoformans* for the following reason: the isolates of *C. neoformans* var. *neoformans* isolated from pigeon droppings, pigeon nests, and soil in the United States have all been of the mating type α (Kwon-Chung and Bennett 1978). If the primary ecological niche of *F. neoformans* is avian droppings, both α and *a* mating types should have been found from these sources. Similarly, *Tremella* (jelly fungi), another fungus phylogenetically related to *F. neoformans,* is found as a saprophyte on dead tree branches, while its haplophase is isolated from various other substrates besides dead tree branches.

Unlike *C. neoformans* var. *neoformans, C. neoformans* var. *gattii* has not been found from a wide range of substrates. It has not been isolated from pigeon or any other bird droppings. Until recently, *C. neoformans* var. *gattii* has only been isolated from clinical specimens collected from humans and animals. An isolation of *C. neoformans* var. *gattii* serotype C from the environment was reported by Denton and DiSalvo (1968), but the isolate was subsequently determined to be an A serotype of the variety *neoformans.* Ellis and Pfeiffer found the natural habitat of *C. neoformans* var. *gattii* serotype B (Ellis and Pfeiffer 1990, Pfeiffer and Ellis 1992). They isolated several strains from wood bark and leaves lying under the canopies of *Eucalyptus camaldulensis* and *E. tereticornis* in Australia. The serotype B isolates of the variety *gattii* were also isolated from materials collected in a koala enclosure in Australia (Pfeiffer and

Ellis 1993). Three strains of *C. neoformans* var. *gattii* isolated from *E. camaldulensis* by Ellis and Pfeiffer were tested in our laboratory and were found to produce fatal infections in mice. When rDNA sequences specific for *C. neoformans* were used to probe RNA extracted from the cultures, the signals of hybridization were equally intense between clinical strains and *Eucalyptus*-originated strains (Kwon-Chung et al. 1992). Attempts to produce the *Filobasidiella* state from these isolates by crossing with type strains of both varieties of *F. neoformans* failed. This was not surprising, since a previous study showed at least 26% of serotype B isolates to be sterile (Kwon-Chung and Bennett 1978). The isolation of *C. neoformans* var. *gattii* from *E. camaldulensis* has epidemiological significance, since the distribution pattern of *E. camaldulensis* corresponded to reported cases of cryptococcosis caused by *C. neoformans* var. *gattii* in Australia (Ellis and Pfeiffer 1990).

Prior to the epidemic of acquired immunodeficiency syndrome (AIDS), cryptococcosis was most commonly reported from patients with leukemia or other kinds of cancer, sarcoidosis, and rheumatoid arthritis (with steroid therapy). Since the early 1980s, AIDS became the leading predisposing condition for cryptococcosis. In the United States, at least 50% of the total cryptococcosis cases reported annually are from AIDS patients, ranking as the fourth most life-threatening infection in these patients. According to one study, in 106 AIDS patients with cryptococcal infection, cryptococcosis was the first manifestation of AIDS in 45% of these patients (Chuck and Sande 1989). The epidemiological studies of the two varieties conducted prior to the AIDS epidemic indicated that infections caused by isolates of *C. neoformans* var. *neoformans* is worldwide in distribution. Infections caused by isolates of *C. neoformans* var. *gattii,* however, were prevalent in tropical and subtropical regions and were rarely found in regions with cold climates (Kwon-Chung and Bennett 1984).

Because cryptococcosis occurs most commonly in AIDS patients, and almost all AIDS patients with cryptococcosis are due to the var. *neoformans* regardless of the geographic location, the overall frequency of infection due to var. *gattii* had diminished drastically since the advent of AIDS. Studies of isolates from two regions previously known as the high-prevalence areas for *C. neoformans* var. *gattii,* Southern California and Brazil, indicated that *C. neoformans* var. *gattii* still causes infection frequently in non-AIDS patients. This indicates that *C. neoformans* var. *gattii* is not disappearing from the environment but that AIDS patients may be more predisposed to infection by *C. neoformans* var. *neoformans* than to var. *gattii,* or they may be less exposed to the source of var. *gattii* than to that of var. *neoformans* (Kwon-Chung et al. 1990).

The holotype strains of *F. neoformans* var. *neoformans* have been proven to be highly virulent for mice. The holotype strains of *F. neoformans* var. *bacillispora* (from clinical source), however, are of low virulence (or avirulent) in mice (Kwon-Chung et al. 1982a). Isolates of *C. neoformans* var. *gattii* from *Eucalyptus,* on the other hand, are as virulent in mice as those of the var. *neoformans* (Kwon-Chung et al. 1992). The low virulence of the isolates of *F. neoformans* var. *bacillispora* from clinical sources was partly explained by their growth rate at 37°C *in vitro. F. neoformans* var. *bacillispora* has a longer doubling time at 37°C than *F. neoformans* var. *neoformans* (Kwon-Chung et al. 1982a). In humans, however, *F. neoformans* var. *bacillispora* causes no less severe infection than *F. neoformans* var. *neoformans.*

Because of the medical importance of *F. neoformans,* various methods for rapid identification were developed. Staib (1962) found that *C. neoformans* was the only tested yeast species to produce dark brown colonies when grown on an agar medium containing niger seed (*Guizotia abyssinica*) extract. Shields and Ajello (1966) developed a medium for selective isolation of *C. neoformans* by incorporating an extract of *G. abyssinica* into an agar medium containing glucose, creatinine, chloroamphenicol, and diphenyl. Pulverer and Korth (1971) found that various *o*- and *p*-diphenols and *o*-triphenols produced results similar to the *G. abyssinica* extract. Strachan et al. (1971) determined that hydroxyl groups in the 3 and/or 4 position of the phenyl ring were common to all compounds which give rise to dark brown pigment. Shaw and Kapica (1972) reported that 3, 4-dihydroxyphenylalanine phenoloxidase found in the cells of *C. neoformans* catalyzes melanin synthesis from *o*-diphenols, but not from tyrosine. According to our experience, niger seed agar gave the most consistent results of melanin formation within 1–5 days compared to chlorogenic acid agar or DOPA agar. Isolates of *C. neoformans* var. *gattii* may, on the average, take longer to produce the color, and the intensity of the pigment may be weaker than that of var. *neoformans.* The concentration of the carbon sources in the medium plays a decisive role for the rapid formation of pigment (Nurudeen and Ahearn 1979). Media containing 1% or higher concentrations of glucose delay the formation of melanin compared with media containing 0.1 or 0.05%. Most isolates of the two varieties produce brown colonies within 1–3 days on niger seed agar with 0.1% glucose. Total elimination of glucose from niger seed agar allowed 60 of 89 isolates to develop the pigment within 12 hours (Palawal and Randhawa 1978). Williamson (1994) found that the transcriptional activity of the structural gene coding for diphenoloxidase (*CNLAC1*) is derepressed when *C. neoformans* is grown in the absence of glucose. Biochemical aspects of phenoloxidase and melanin synthesis in *C. neoformans* were studied (Polacheck et al. 1982, Polacheck and Kwon-Chung 1988, Williamson 1994) and were found to be similar to that found in melanoma cells, except that it starts from DOPA instead of tyrosine. The ability to form melanin was shown to be one of the important virulence factors in *C. neoformans.* The mutants lacking phenoloxidase activity were found to have lost the ability to cause fatal infection in mice, and their virulence was

restored when the mutants reverted to show phenoloxidase activity (Rhodes et al. 1982, Kwon-Chung and Rhodes 1986). Employing molecular methods, Salas et al. (1996) confirmed that the *CNLAC1* gene plays a significant role in virulence for mice.

Several one-step diagnostic media to distinguish the isolates of the two varieties have been developed on the bases of their biochemical differences. Only *C. neoformans* var. *gattii* grows on CGB medium and produces a blue color, because it can utilize glycine as the sole source of carbon and nitrogen, in addition to being resistant to L-canavanine (Kwon-Chung et al. 1982b). Only *C. neoformans* var. *gattii* isolates grow on D-proline agar because they can utilize D-proline as a nitrogen source (Dufait et al. 1987). *C. neoformans* var. *gattii* grown on an agar medium containing ethylenediaminetetraacetate (EDTA) produces no urease activity in rapid urea broth, because the enzyme is sensitive to EDTA. The urease of *C. neoformans* var. *neoformans*, however, is resistant to EDTA (Kwon-Chung et al. 1987).

Most isolates of the two varieties are sensitive to $\leqslant 12\,\mu g/ml$ of cycloheximide and, therefore, agar media containing cycloheximide, such as mycosel agar, should not be used for isolation. Isolates of *C. neoformans* var. *gattii* grow very slowly at 37°C, and the primary isolation tubes or plates should be incubated at 30–35°C. The optimum growth temperature for both varieties is 32°C.

A mechanism of basidiospore formation, similar to *Filobasidiella* species (Kwon-Chung 1976b), is not found in the other genera belonging to the Filobasidiales, and the author is not aware of any comparable situation in the basidiomycetes in general. Species of *Schroeteria* previously considered to belong the Ustilaginales produce spores in chains, but they are produced by acropetal budding (Zundel 1953) rather than basipetal budding as in the case of *Filobasidiella*. Recently, Nagler et al. (1989) concluded that the genus *Schroeteria* should be excluded from the Ustilaginales because the species of *Schroeteria* possessed septal pores, Woronin bodies, and cell walls characteristics of Ascomycetes.

The cytology of basidiospore formation by *F. neoformans* partly resembles that of *Ustilago* species (Alexopoulos and Mims 1979). In *Ustilago maydis,* the nuclei of the first meiotic product divide mitotically and daughter nuclei migrate into the basidiospores (sporidia), while parent nuclei remain in the promycelium. Unlike *Filobasidiella* species, however, the four daughter nuclei are separated from each other by septa of the promycelium, and basipetal chains of sporidia are not produced.

Progress on the molecular biology of *F. neoformans* has been made since a method for spheroplast formation and the isolation of high molecular-sized DNA using Novozyme was described (Rhodes and Kwon-Chung 1985). Mitochondrial restriction patterns of the two varieties have shown a great deal of polymorphism (Varma and Kwon-Chung 1989). Numerous genes, including rDNA (Restrepo and Barbour 1989, Kwon-Chung and Chang 1994) *URA5* (Edman and Kwon-Chung 1990), *TRYP1* (Perfect et al. 1992), *DHFR* (Sirawaraporn et al. 1993), *HIS3* (Parker et al. 1994), *NMT* (Lodge et al. 1994), *CAP59* (Chang and Kwon-Chung 1994), *CNLAC1* (Williamson 1994), *GAL7* (Wickes and Edman 1995) and various others (Wickes et al. 1994) were cloned from *F. neoformans* var. *neoformans.* The transformation was accomplished in both varieties by using plasmids containing the *URA5* gene or *ADE2* gene as the selection markers (Edman and Kwon-Chung 1990, Toffaletti et al. 1993, Lodge et al. 1994). The *CAP59* and the *CAP64* genes responsible for synthesis of the polysaccharide capsule have been proven by molecular methods to be essential for virulence in *C. neoformans.* Deletion of these genes in a wild type strain resulted in the loss of capsule as well as the loss of virulence in mice. Conversely, acapsular, avirulent mutants with lesions in the *CAP59* and the *CAP64* loci regained capsule and virulence when the strains were complemented with the genes (Chang and Kwon-Chung 1994, Chang et al. 1996).

Malloch et al. (1978) isolated a filamentous fungus from a dead spider and described it as *Filobasidiella arachnophila.* Samson et al. (1983) found that *F. arachnophila* is identical to a fungus previously known as *Aspergillus depauperatus* Petch. Since *A. depauperatus* morphologically resembled *Filobasidiella* rather than *Aspergillus,* Samson et al. (1983) described a new combination *F. depauperata* (Petch) Samson, Stalpers and Weijman. Like *F. neoformans, F. depauperata* produces four spore chains on the apex of the basidium; however, *F. depauperata* lacks clamp connections and haustorium-like structures in the hyphae and produces no yeast cells in any part of its life cycle. *F. depauperata* also lacks the key biochemical markers of *F. neoformans,* namely the ability to hydrolize urea, to oxidize diphenols, to assimilate inositol, and to synthesize starch-like compounds (Khan et al. 1981a). Despite these differences, the phylogenetic trees of Heterobasidiomycetes constructed on the bases of the 18S, 5.8S and 26S rDNA sequences (Kwon-Chung et al. 1995, Mitchell et al. 1992, Guého et al. 1993) indicate that *F. depauperata* is most closely related to *F. neoformans* and that both species have evolved from the *Tremella*-lineage. Ultrastructural studies of *F. depauperata* support its proposed *Tremella*-lineage (Kwon-Chung et al. 1995). The spindle pole bodies of *F. depauperata* and *F. neoformans* are nearly identical to those reported for *Tremella globosa* (Berbee and Wells 1988, Mochizuki et al. 1987). Hyphal septa of *F. depauperata* demonstrate dolipores with parenthesomes consisting of the separate cupulate vesicles typical of *Tremella* species. Some basidia of *F. depauperata* show evidence of partial longitudinal apical division (Kwon-Chung et al. 1995). Such partially septated basidia were seen in *Rhynchogastrema coronata* of the Tremellales (Metzler et al. 1989). *F. depauperata,* however, is not discussed in this chapter because it lacks a yeast stage.

83. *Filobasidium* Olive

K.J. Kwon-Chung

Diagnosis of the genus

Asexual reproduction by budding of globose, ovoidal, ellipsoidal or elongated cells. Pseudomycelium and true mycelium may be produced. Growth on solid media is white, cream-colored, or slightly pinkish, and often mucoid.

Sexual reproduction is heterothallic (Fig. 327), and following mating, the mycelium produces lateral or terminal holometabasidia. Basidiocarps are absent. Sessile basidiospores are produced terminally on the apex of the metabasidium in a petal-like whorl, giving the basidium a flower-like appearance in an apical view. Clamp connections are present at cross walls, and the septa contain typical dolipores with or without parenthesomes.

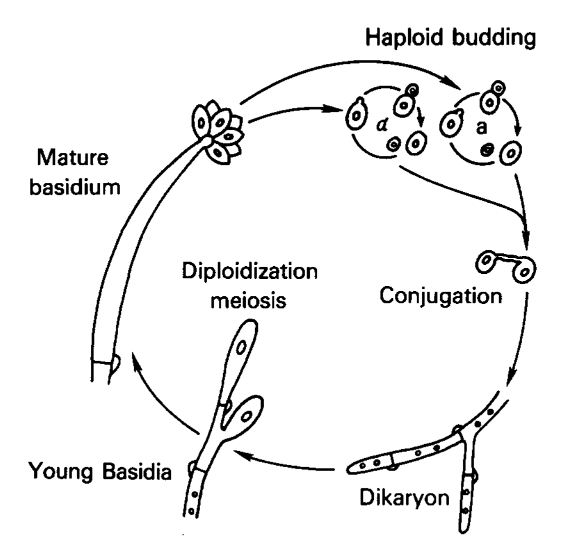


Fig. 327. Life cycle of *Filobasidium*.

Fermentation may be present. D-Glucuronate, *myo*-inositol and nitrate are sometimes assimilated. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-9 or 10 are formed. Xylose is present in whole-cell hydrolyzates.

Type species

Filobasidium floriforme Olive

Species accepted

1. *Filobasidium capsuligenum* (Fell, Statzell, I.L. Hunter & Phaff) Rodrigues de Miranda (1972)
2. *Filobasidium elegans* Bandoni & Oberwinkler (1991)
3. *Filobasidium floriforme* Olive (1968)
4. *Filobasidium globisporum* Bandoni & Oberwinkler (1991)
5. *Filobasidium uniguttulatum* Kwon-Chung (1977)

Key to species

See Table 68.

1.	a Basidiospores less than 10 μm long → 2		
	b Basidiospores more than 10 μm long → 3		
2(1).	a Basidia with up to 10 reniform to ovoidal (3–5×4–9 μm) basidiospores per basidium	*F. uniguttulatum*:	p. 667
	b Basidia with up to 5 subglobose to apiculate-ovoidal (6–8.5×8–9 μm) basidiospores per basidium	*F. globisporum*:	p. 667
3(1).	a Basidia more than 200 μm long; basidiospores 6–9 μm wide	*F. floriforme*:	p. 666
	b Basidia mostly less than 140 μm long; basidiospores mostly less than 6 μm wide → 4		

4(3). a Basidiospores mostly allantoid or reniform .... *F. elegans*: p. 665
b Basidiospores ellipsoid to fusiform .... *F. capsuligenum*: p. 664

Table 68
Key characteristics of species in the genus *Filobasidium*

Species	Glucose fermentation	Assimilation			Growth at 37°C	Basidiospores
		Lactose	Melezitose	Nitrate		
Filobasidium capsuligenum	+	–	–	–	w/–	ellipsoidal to fusiform (4–6)×(9–13) µm
F. elegans	–	–	–	–	–	allantoid to reniform (4.5–7)×(8.5–15) µm
F. floriforme	–	+	+	+	–	ellipsoidal to ovoidal (6–9)×(10–17) µm
F. globisporum	–	s	–	–	–	subglobose to apiculate-ovoidal (6–8.5)×(8–9) µm
F. uniguttulatum	–	–	+	–	–	reniform to ovoidal (3–5)×(4–9) µm

Systematic discussion of the species

83.1. *Filobasidium capsuligenum* (Fell, Statzell, I.L. Hunter & Phaff) Rodrigues de Miranda (1972)

Anamorph: *Candida japonica* Diddens & Lodder

Synonyms:

Leucosporidium capsuligenum Fell, Statzell, I.L. Hunter & Phaff (1969)
Torulopsis capsuligena (as *T. capsuligenus*) van der Walt & van Kerken (1961b)
Candida capsuligena (van der Walt & van Kerken) van Uden & H.R. Buckley (1970)
Torulopsis alba (as *T. albus*) Saito & Oda (1934)
Candida japonica Diddens & Lodder (1942)
Azymoprocandida japonica (Diddens & Lodder) Novák & Zsolt (1961)

Growth in 2% malt extract: After 3 days at 25°C, the haploid cells are subglobose, ovoidal, ellipsoidal to elongated, (2.5–10.7)×(4.0–17.8) µm, single or in pairs. A thin ring and a little sediment are formed. CBS 4736 produced a heavier film than the remaining two strains tested. After one month, there is a moderate to thick slimy ring and a heavy sediment.

Growth on 2% malt agar: After three days, the cells are similar in size and shape (Fig. 328A) to those formed in malt extract, except that elongated pseudomycelial cells occur. The streak culture is white to cream-colored, and the texture varies from dry to mucoid. The colony margin is entire and smooth or with tufts of mycelium. After 3 weeks, mating type α and a strains show differences in colony morphology. Strains of α type are mucoid, glistening, cream-colored, with a raised surface and smooth margin; mycelium is lacking. Colonies of mating type a have a soft, moist or dry texture and are not mucoid. The margin of the colony is undulate and heavily myceliated. The mycelium consists of branched, septate hyphae.

Dalmau plate culture on corn meal agar: Pseudomycelium or mycelium is absent in mating type α, while true mycelium is present in mating type a. Occasionally, a few short chains of undifferentiated, globose cells may be present.

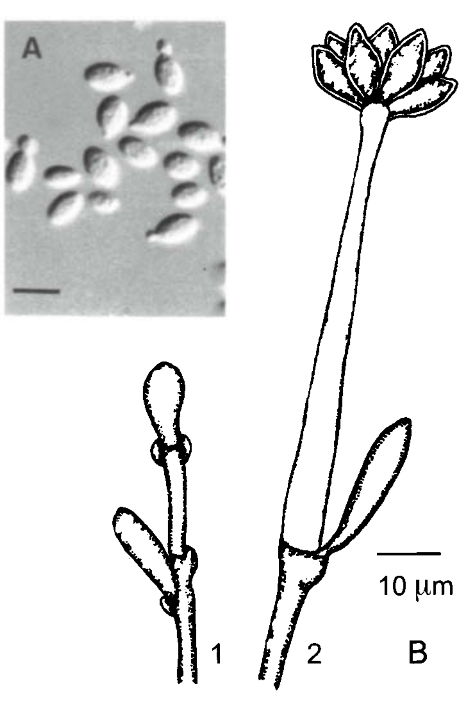


Fig. 328. *F. capsuligenum*. (A) Yeast cells grown on malt agar for 3 days. Bar = 10 µm. (B1) Young basidium. (B2) Mature basidium.

Life cycle: On corn meal agar, conjugation occurs between two mating types within 12 hours of mixing at room temperature. Dikaryotic hyphae with clamp connections grow out from a conjugated pair within 48 hours. After 3 weeks, slender, holometabasidia measuring (3.0–6)×(70–140) µm are produced laterally or terminally on the dikaryotic mycelium (Fig. 328B1); five to nine sessile ellipsoidal to fusiform basidiospores, (4–6)×(9.5–13) µm, bud on the apices of the metabasidia (Fig 328B2). Occasionally, single isolates may produce mycelium and basidial structures without apparent mating.

Fermentation:

Glucose	+	Lactose	–
Galactose	–	Trehalose	–
Raffinose	–	Sucrose	–
Maltose	v		

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	v
Galactose	s	Methanol	–
L-Sorbose	–	Ethanol	w
Sucrose	+	Glycerol	+/w
Maltose	+	Erythritol	–
Cellobiose	+/w	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+/w
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	s
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	v	Succinate	s
L-Arabinose	v	Citrate	+/w
D-Arabinose	v	Inositol	+
D-Ribose	v	Hexadecane	v
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	+
D-Saccharate	–	Urease	+
D-Glucuronate	+	Nitrite	–
L-Lysine	+	Growth at 30°C	+
Ethylamine	+	Growth at 37°C	w/–
50% Glucose (w/w)–yeast extract agar	–		

Vitamin requirement: Thiamine or thiamine + biotin.
Co-Q: 10 (Yamada and Kondo 1972a).
Mol% G + C: 49.8 (T_m: Nakase and Komagata 1971c).

Origin of the strains studied: CBS 1906 (*a* type) described as *Torulopsis albus* by Saito and Oda (1934) and isolated from sake-moto; CBS 4381 (*a* type) from cider by Beech, Bristol; CBS 4736, type culture of *Leucosporidium capsuligenum* (*α* type) from a wine cellar and described as *Torulopsis capsuligenus* (van der Walt and van Kerken 1961b); CBS 4173 from grapes in Germany; CBS 6122 from forest soil, Braunschweig, Germany.

Complementary mating types: CBS 1906 (*a*) and CBS 4763 (*α*).

Type strain: CBS 1906.

83.2. *Filobasidium elegans* Bandoni & Oberwinkler (Bandoni et al. 1991)

Growth on 2% malt agar: The cells multiply by unipolar budding, and are predominantly ellipsoidal, (2.5–5) × (3.5–7.5) μm, but a few cells may be elongate or spheroidal to subspheroidal. Colonies are mucoid and grayish-white to pale cream in color.

Life cycle: Sexuality of *F. elegans* has not been defined. Thin-walled, hyaline hyphae (2–3 μm diameter) with clamp connections and haustorial branches produce clavate, thin-walled probasidia singly or arranged in a loose to relatively thin compact hymenium-like aggregation. Each probasidium elongates to become a tubular basidium with an abruptly swollen apex (Fig. 329); mature basidia (65–165 μm long) have thick walls, especially near the apex (Fig. 329B). The diameter of basidia

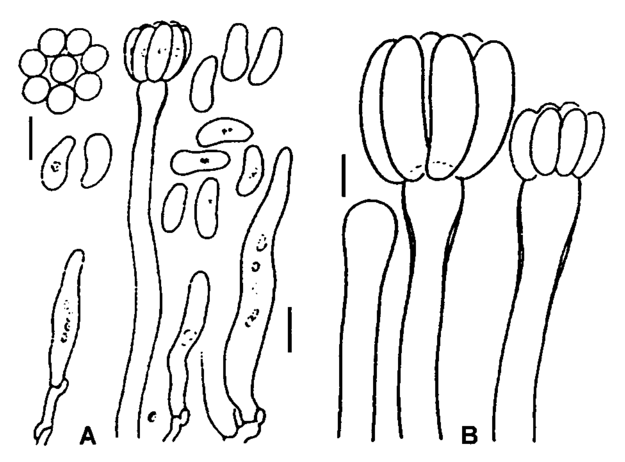


Fig. 329. *F. elegans* (Bandoni et al. 1991). (A) Probasidia, a mature basidium with basidiospores and liberated basidiospores (allantoid or reniform). Top view of basidiospores while they are on the basidium (circular arrangement shown on the top left corner). Bars = 10 μm. (B) Basidia with basidiospores. Bar = 5 μm.

is 3.5–5.5 μm in the middle portion and 6–6.5 μm at the apex bearing basidiospores. Basidia bear 5 to 8 sessile basidiospores that leave minute scars of attachment on the surface of the apex after secession. After the spore secession, basidia collapse inward. Basidiospores, (4.5–7.5 × 8.5–15) μm, which are reniform or infrequently ellipsoidal, are usually arranged in a circle of 4 or 6 around a single central basidiospore (Fig. 329A). The circular arrangement is usually retained for a short time after release. Basidiospores germinate by budding.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	s	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	w	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	s	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	+
D-Saccharate	–	Nitrite	–
D-Glucuronate	–	Thiamine-free	–
Creatinine	–	0.01% Cycloheximide	–
L-Lysine	–	1% Acetic acid	–
Ethylamine	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	–

Co-Q: Not determined.
Mol% G + C: Not determined.

Origin of the strain studies: CBS 7638, 7639, 7640, and 7641 were all isolated from dead inflorescence of *Yucca brevifolia* collected near Gorman, Los Angeles County, California.

Holotype: *F. elegans* on weathered inflorescence scape of *Yucca brevifolia*, collected 10 miles east of Gorman, Los Angeles County, California, was deposited in the herbarium, National Fungus Collection (BPI 118718).

Comments: *Filobasidium elegans* was described by Bandoni et al. (1991) from a fungus collected on a weathered inflorescence scape of various *Yucca* species in California. The anamorph of *F. elegans* is a budding, yeastlike fungus that produces mucoid colonies with a grayish-white to pale cream color. The physiology of *F. elegans* was not characterized by the authors and the carbon assimilation profile was extracted from the CBS data base.

83.3. *Filobasidium floriforme* Olive (1968)

Growth in 2% malt extract: After 3 days at 25°C, the haploid cells are subglobose, ovoidal, or ellipsoidal to long-ovoidal, (2.5–6)×(3–8) µm, single, in pairs, or in small clusters. A small amount of sediment is present. After one month, a moderate ring and sediment are present.

Growth on 2% malt agar: Cell morphology is similar to that in malt extract (Fig. 330A). The streak culture is dull-white to cream-colored and highly mucoid after one month. The colony margin is entire with no mycelium. On corn meal agar, growth is thin, and some strains bear a tinge of pink color.

Dalmau plate culture on corn meal agar: Pseudo- and true mycelium are absent in the haploid state.

Life cycle: Conjugation occurs when cells of two compatible mating types are mixed on V8 juice or hay infusion agar. Soon after conjugation, dikaryotic hyphae with clamp connections are produced from the conjugant.

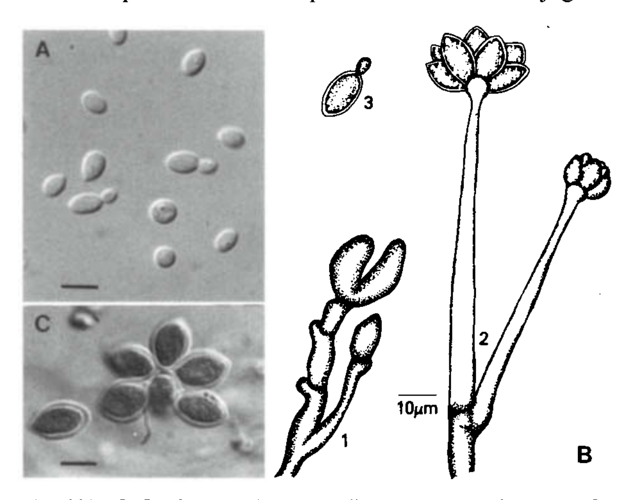


Fig. 330. *F. floriforme.* (A) Yeast cells grown on malt extract for 3 days. Bar = 10 µm. (B1) Young basidium. (B2) Mature basidium. (B3) Germinating basidiospore. (C) Seven basidiospores released from a single basidium. Bar = 10 µm.

The hyphal septum contains a dolipore but not parenthesomes. Long slender holometabasidia, (3.2–7)×(57–212) µm, arise mostly from branching clusters of hyphae. The thin-walled young metabasidia are slightly broader (Fig. 330B1) than the hyphae bearing them and contain a denser cytoplasm. Six to eight ellipsoidal to ovoidal basidiospores, (6–9)×(10.5–16.5) µm, are formed directly from the expanded apex (Fig 330B2,C) of the basidia. Basidiospores produce blastoconidia (Fig. 330B3), which continue to bud profusely and form yeast colonies. Occasional isolates have been shown to produce a complete sexual state without mating. Karyogamy and meiosis occur in metabasidia.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+/w	Methanol	–
L-Sorbose	–	Ethanol	s
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+/s
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	s
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+/w
Melezitose	+	Salicin	+
Inulin	v	D-Gluconate	s
Soluble starch	+	DL-Lactate	–
D-Xylose	+/w	Succinate	+/w
L-Arabinose	+/w	Citrate	+/w
D-Arabinose	+/w	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	+
D-Saccharate	+	Urease	+
D-Glucuronate	+	Nitrite	–
Creatinine	–	Thiamine-free	–
L-Lysine	v	Growth at 35°C	+
Ethylamine	+	Growth at 37°C	–
50% Glucose (w/w)–yeast extract agar	+		

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G + C: Not determined.

Origin of the strains studied: Plume grass (*Erianthus giganteus*) (3); *Hibiscus siriaca* (2); Yucca (*Yucca rupicola*) (3).

Complementary mating types: CBS 6241 (*α*) and CBS 6242 (*a*).

Holotype: Herbarium material SC29 was designated as the type by Olive and deposited at the New York Botanical Garden in the Bronx, New York.

Extype strain: CBS 6241, isolated from *Hibiscus siriaca.*

Comments: Although the haploid state of *F. floriforme* is phenotypically similar to *Cryptococcus albidus,* the type strain and many other *C. albidus* strains, including

every variety, failed to mate with α or a mating types of *F. floriforme.*

83.4. *Filobasidium globisporum* Bandoni & Oberwinkler (Bandoni et al. 1991)

Growth on 2% malt agar: The cells, (2.5–5.5×3–9) μm, multiply by budding, and are spheroidal to ellipsoidal or subcylindrical, occasionally with a small extension on one or both ends. The colonies are mucoid and grayish-white to cream in color.

Life cycle: The sexual pattern of *F. globisporum* has not been defined. Hyphae (2–3 μm in diameter) are thin walled with clamp connections and haustorial branches. Basidia are subtended by clamps and produced in dense white patches that are hymenium-like. Mature basidia (35–122 μm long) are 2.5–3.5 μm wide in the middle portion and expanded to 4–5.5 μm apically, and bear 3–5 basidiospores on coarse tubercles (Fig. 331). Some basidia produce 1–3 short lateral branches, which may be sporogenous or remain sterile (Fig. 331A). Basidial walls are thicker immediately below the swollen apex, which shows coarse tubercles that mark the sites of spore attachment. Basidiospores are globose (6–8.5 μm in diameter) or apiculate-ovoidal or broadly ovoidal in side view (6–8.5×8–9 μm). Basidiospores germinate by budding.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	s	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	w	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+/s	Citrate	v
D-Arabinose	–	Inositol	s
D-Ribose	–	Hexadecane	n
L-Rhamnose	s	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
D-Saccharate	–	Nitrite	–
D-Glucuronate	v	Thiamine-free	–
Creatinine	–	0.01% Cycloheximide	–
L-Lysine	–	Growth at 25°C	+
Ethylamine	–	Growth at 37°C	–
Starch formation	+		

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strains studied: CBS 7642, 7643, 7644, and 7645 were isolated from weathered leaf of *Yucca brevifolia* collected 10 miles east of Gorman, Los Angeles County, California.

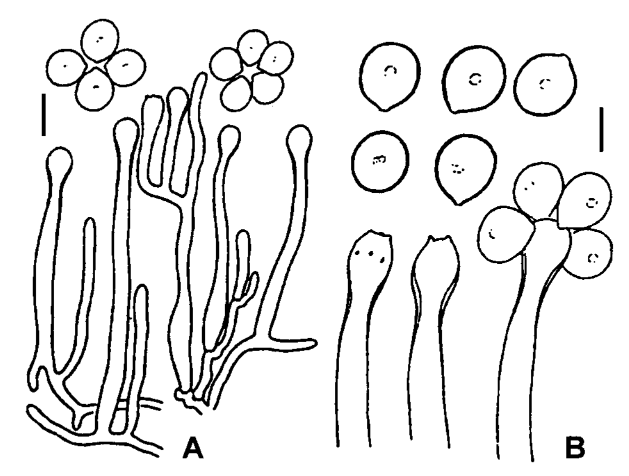


Fig. 331. *F. globosporum.* (A) Fertile hyphae with clamps, young basidia and basidiospores. (B) Mature basidia and basidiospores (Bandoni et al. 1991). Bars = 10 μm.

Holotype: *F. globisporum* on weathered leaves of *Yucca brevifolia,* collected 10 miles east of Gorman, Los Angeles County, California, was deposited at the herbarium, National Fungus Collection (BPI 1108721).

Comments: Bandoni et al. (1991) described *F. globisporum* on the basis of a fungus found on weathered leaves of *Yucca brevifolia* growing in California and Nevada. The anamorph of *F. globisporum* is a budding, yeastlike fungus that produces mucoid colonies with grayish-white to cream color. The description of *F. globisporum* is an excerpt from the original paper by Bandoni et al. (1991), and the assimilation profile was extracted from the CBS database.

83.5. *Filobasidium uniguttulatum* Kwon-Chung (1977b)

Anamorph: *Cryptococcus uniguttulatus* (Zach) Phaff & Fell

Synonyms:

Eutorulopsis uniguttulata Zach (Wolfram & Zach 1934b)
Cryptococcus uniguttulatus (Zach) Phaff & Fell (1970)
Cryptococcus neoformans (Sanfelice) Vuillemin var. *uniguttulatus* (Zach) Lodder & Kreger-van Rij (1952)

Growth in 2% malt extract: After 3 days at 25°C, the haploid cells are globose to ovoidal, single, in pairs, or in small clusters, (3.0–5.5)×(3.5–7.0) μm, rarely up to 9 μm long. A thin ring and a small amount of sediment may be present. After one month, there is a moderate ring and a moderate amount of sediment.

Growth on 2% malt agar: Shape and size of the cells are similar to those grown in malt extract (Fig. 332A). After one month at 25°C, the streak culture is white to cream-colored with an entire margin. Colony texture is smooth, soft, and semiglossy.

Dalmau plate culture on corn meal agar: Pseudo- and true mycelium are not formed in the haploid phase.

Life cycle: On V8 juice or hay infusion agar, cells

produce conjugation tubes, (1–5)×(1.5–20) μm, within 2–3 days after mixing of two compatible mating types. The conjugation tube is usually sinuous and narrower than the dikaryotic hyphae, which arise from the conjugated cells. The dikaryotic hyphae bear clamp connections at each septum. Within 7–10 days, mature metabasidia appear in the margin of mated cultures. Young metabasidia are thin-walled and broader than the hyphae from which they originate. They develop singly or in groups of two to four on the side of hyphae at the clamp or terminally on the hyphae (Fig. 332B). Blastospores are occasionally produced laterally on the hyphae. Metabasidia are elongated, (4–6)×(30–90) μm; they taper apically and expand abruptly to produce a globose apex (3–5 μm diameter) (Fig. 332D) with four to ten sessile terminal basidiospores (Fig. 332C). The basidiospores bud directly from the apex. The spores are ovoidal to kidney-shaped, (3–5)×(4–9) μm. Infrequently, haustorioid branches are produced from the clamp of the dikaryotic hyphae. The liberated basidiospores reproduce by budding and form yeast colonies. Although a detailed cytological study was not carried out, analysis of single basidiospores indicated that meiosis occurred in the metabasidium.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	w
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	v
Trehalose	+/w	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	+/w
Raffinose	s	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	+/w
Inulin	+	D-Gluconate	s
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	v
L-Arabinose	+/w	Citrate	v
D-Arabinose	w/–	Inositol	+
D-Ribose	–	Hexadecane	w
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
D-Saccharate	–	Urease	+
D-Glucuronate	+	Nitrite	–
Creatinine	–	Thiamine-free	–
L-Lysine	+/w	Growth at 30°C	+
Ethylamine	–	Growth at 35°C	–
50% Glucose (w/w)–yeast extract agar	–		

Co-Q: 9 (Yamada and Kondo 1972a).

Mol% G+C: 51.5 (T_m: Storck et al. 1969, Nakase and Komagata 1971c).

Origin of the strains studied: Five strains were from clinical specimens: CBS 1730 (*a*) and CBS 1727 (*α*) from diseased nails; CBS 2770 (*α*) from white spots in the mouth; CBS 2994 (*α*) from a bronchus; and CBS 4257 (*α*) from sputum. Five strains were single basidiospore cultures obtained by crossing CBS 1730 and CBS 1727.

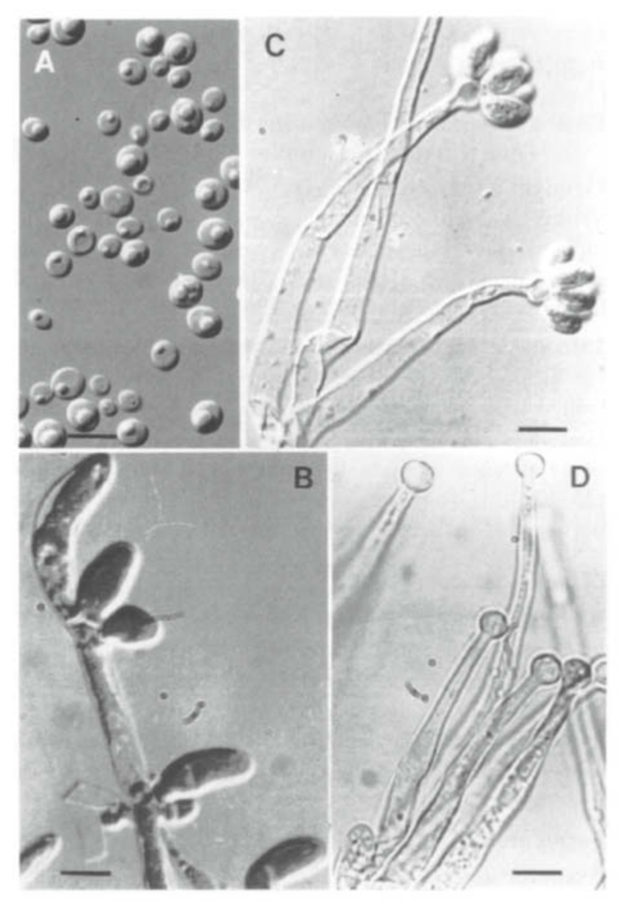


Fig. 332. *F. uniguttulatum.* (A) Yeast cells grown on malt extract agar for 3 days. (B) Young basidia. (C) Mature basidia. (D) Basidia after the release of basidiospores. Bars = 10μm.

Complementary mating types: CBS 1727 (*α*) and CBS 1730 (*a*).

Type strain: CBS 1730.

Comments: Yamada and Kondo (1972a) studied the Co-Q system of eight species and three varieties in the genus *Cryptococcus.* They found that, except for *C. uniguttulatus,* all had the Q-10 system. The known teleomorphs of species with the Q-10 system belonged either to *Filobasidiella* or *Filobasidium.* The coenzyme Q of *C. uniguttulatus,* the anamorph of *F. uniguttulatum,* however, was Q-9. The question remains whether the isolate that Yamada and Kondo studied produces the *F. uniguttulatum* teleomorphic state. Although *F. uniguttulatum* has been isolated from diseased nails (human) or other clinical specimens, it has not been documented to cause invasive disease.

Comments on the genus

Fell et al. (1969) described the sexual state of *Candida*

japonica (*Candida capsuligena*) as *Leucosporidium capsuligenum*. Basidiospores and basidia were not mentioned, but terminal teliospores connected to the hyphae by clamp connections were described. These were later found to be the initial stage of metabasidium formation. Rodrigues de Miranda (1972) observed the complete sexual life cycle of the species and redescribed the species as *F. capsuligenum*.

Physiologically, *F. capsuligenum* differs from other members of the genus by the ability to ferment glucose and maltose. The extracellular amylolytic systems of *F. capsuligenum* were studied in detail by De Mot and Verachtert (1985). The purified α-amylase (1,4-α-D-glucanglucanhydrolase) and two forms of glucoamylase (1,4-α-D-glucanglucohydrolase). The molecular weight of the α-amylase was 64 000, while that of glucoamylase was 60 000. The optimum temperature and pH for α-amylase are 50°C and 5.6, respectively. Although the two forms of glucoamylase had identical molecular weight, their optimum temperature and pH ranges were different.

Ultrastructurally, *F. capsuligenum* differs from *F. floriforme* by having cone-shaped vesicular parenthesomes, while the latter species lacks the parenthesome (Moore and Kreger-van Rij 1972). Such parenthesomes were also observed in *Tremella* species (Khan 1976). *F. uniguttulatum* differs form *F. floriforme* by the inability to assimilate nitrate and lactose. *Cryptococcus uniguttulatus*, the asexual state of *F. uniguttulatum*, was first described as *Eutorulopsis uniguttulata* by Zach (Wolfram and Zach 1934b). Lodder and Kreger-van Rij (1952) redescribed this yeast as *Cryptococcus neoformans* var. *uniguttulatus* on the basis of morphological and physiological similarities with *C. neoformans*, but with reduced capsule formation. In 1961, Kreger-van Rij recognized that isolates of *C. neoformans* assimilated galactitol as a sole carbon source and were able to grow at 37°C, whereas the variety was not able to do so. An additional difference in creatinine utilization was found between the species and variety by Staib (1963). In 1970, Phaff and Fell reestablished the species status for the variety as *C. uniguttulatus*. With the discovery of the *Filobasidium* state, it is now clear that *C. uniguttulatus* is phylogenetically closer to *Candida japonica* and *Cryptococcus albidus* than to *C. neoformans*. Physiological and morphological characteristics of *C. albidus* fit well with the asexual state of *F. floriforme*; however, we failed to demonstrate successful mating between the type strains of the *C. albidus* varieties with the tester of *F. floriforme*.

The genus *Filobasidium* can readily be separated from *Filobasidiella* by differences in the morphology of their metabasidia. The intergeneric homology of 25S rRNA studied by relative binding of unlabeled 25S rRNA from *F. capsuligenum* and *F. uniguttulatum* with a ^{3}H-labeled cDNA probe of *F. neoformans* showed a minimal difference of 16.11 relative binding% (Baharaeen and Vishniac 1984). The 18S and 25S rRNA sequence analysis supported such observations. Guého et al. (1989) found differences in at least 32 positions within the region of 350 nucleotides in the 25S rRNA and 17 positions within the region of 332 nucleotides in the 18S rRNA.

In 1991, Bandoni et al. described two new species of *Filobasidium*, *F. elegans* and *F. globisporum*, both isolated from weathered inflorescences and leaf parts of several *Yucca* species collected in California, Nevada, and Texas. Bandoni et al. (1991) did not characterize the yeast phases of the species. The description of the two species indicated that they can be distinguished from the three previously known species by the shape and size of the basidiospores and the length of the basidia. Elucidation of their life cycle, biochemical characteristics of the yeast phase, and nucleotide sequence of rRNA are needed to understand the relationship of the two species with the remaining *Filobasidium* species.

84. *Leucosporidium* Fell, Statzell, I.L. Hunter & Phaff

A. Statzell-Tallman and J.W. Fell

Diagnosis of the genus

Growth on solid media is white to cream-colored, often mucoid. Visible carotenoid pigments are not present. Both yeast and mycelial phases may occur. Yeast phase cells are ovoidal to elongate and reproduce by budding; a pseudomycelium may form. The mycelial phase is characterized by true mycelium with or without clamp connections and the formation of terminal and intercalary teliospores. The septal structure is a simple pore. Ballistospores and ballistoconidia are not present.

Sexual mechanisms, although not completely investigated, include unifactorial (bipolar) and bifactorial (tetrapolar) systems. In these heterothallic systems, a dikaryotic mycelium results from the conjugation of a compatible mating pair. A clamp connection is usually present at each cross wall. Intercalary and terminal teliospores develop. Karyogamy takes place in the teliospore (probasidium) which germinates to produce a metabasidium (promycelium) that is septate, usually with four cells (phragmometabasidium). Meiosis takes place during germination and the resulting haploid basidiospores are lateral and terminal on the phragmometabasidia.

Teliospores can be produced, in the apparent absence of mating, by two mechanisms. Uninucleate yeast cells of *L. antarcticum* and *L. scottii* produce uninucleate teliospore-bearing hyphae that lack true clamp connections. In contrast, the uninucleate yeast cells of *L. fellii* are diploid, reduction division takes place with the formation of dikaryotic teliospore-bearing hyphae with clamp connections; karyogamy occurs in the teliospores, which germinate to a metabasidium with diploid basidiospores.

Glucose is not fermented. Nitrate is assimilated. Diazonium blue B and urease reactions are positive. *Myo*-inositol is not assimilated. Starch-like compounds are not produced. Coenzymes Q-9 and Q-10 are present. Xylose is absent from cell hydrolyzates.

Type species

Leucosporidium scottii Fell, Statzell, I.L. Hunter & Phaff

Species accepted

1. *Leucosporidium antarcticum* Fell, Statzell, I.L. Hunter & Phaff (1969)
2. *Leucosporidium fellii* Giménez-Jurado & van Uden (1989)
3. *Leucosporidium scottii* Fell, Statzell, I.L. Hunter & Phaff (1969)

Key to the teliospore-forming white yeasts

See Table 69.

1. a Glucose fermented → 2
   b Glucose not fermented → 3

2(1). a Nitrate assimilated .... *Mrakia frigida*: p. 676
   b Nitrate not assimilated .... *Cystofilobasidium lari-marini*: p. 652

3(1). a Melezitose assimilated .... *L. scottii*: p. 673
   b Melezitose not assimilated → 4

4(3). a L-Rhamnose assimilated .... *L. fellii*: p. 671
   b L-Rhamnose not assimilated → 5

5(4). a Nitrate assimilated .... *L. antarcticum*: p. 671
   b Nitrate not assimilated .... *Rhodotorula fujisanensis*: p. 812

Table 69
Key characters of the teliospore-forming white yeasts

Species	Glucose fermentation	Assimilation		
		Melezitose	L-Rhamnose	Nitrate
Cystofilobasidium lari-marini	+	+	v	–
Leucosporidium antarcticum	–	–	–	+
L. fellii	–	–	+	+
L. scottii	–	+	+	+
Mrakia frigida	+	v	v	+
Rhodotorula fujisanensis	–	–	–	–

Systematic discussion of the species

84.1. *Leucosporidium antarcticum* Fell, Statzell, I.L. Hunter & Phaff (1969)

Growth in 5% malt extract: After 3 days at 12°C, the cells of self-sporulating strains are ovoidal, (2–4)×(5–22) µm; haploid, heterothallic cells tend to be shorter and wider and are (3–6)×(3–10) µm. Both types of cells are single, in pairs and short chains. There is a light sediment. After one month there is a light ring and a heavy sediment.

Growth on 5% malt agar: After 3 days at 12°C, the cells are approximately the same size as in malt extract. The cells may have a large capsule and refractile inclusions. Growth is cream-colored, smooth, mucoid, runny and glistening; the border is entire. Pseudo- and true mycelium may be present. There is little change after one month.

Dalmau plate culture on corn meal agar: Self-sporulating strains have two types of mycelium: short branched chains of pseudomycelium and long branched chains of true mycelium. After 3 weeks of growth, teliospores develop on the true mycelium and measure (4–6)×(5–8) µm; they occur terminally, singly and occasionally in pairs. The teliospores increase in size and at 7 weeks are (5–8)×(5–10) µm, terminal, intercalary, single and in short chains of two to three. The teliospores usually have one or two bud-like structures that may represent either vestigial clamp connections or undeveloped sister teliospores. Heterothallic strains form true and pseudomycelium.

Life cycle: Self-sporulating strains: septate uninucleate hyphae with teliospores, lacking clamp connections, develop directly from single cells in the absence of mating. Teliospores from a culture grown on corn meal agar at 5°C, germinated on 2% agar after soaking in distilled water. The highest number of germinating teliospores occurred after 3 months; duration of the individual steps varied. For example, growth on corn meal agar for 2 weeks followed by soaking for 10 weeks or growth for 10 weeks with soaking for 2 weeks. Germination then occurred within one week on 2% agar. When the various steps were performed at 5°C, the cells produced less interfering polysaccharides than at 12°C. The metabasidium was four-celled with lateral and terminal basidiospores. Ploidy of the basidiospores has not been determined.

Heterothallism: compatible mating strains *A1* and *A2* mixed on corn meal agar formed spherical to ovoidal, (7–10)×(8–13) µm, teliospores on clamp-bearing mycelium after 3 weeks at 12°C. Teliospore germination occurred after soaking 2-month-old teliospores in distilled water for one month followed by one week on 2% agar. Basidiospores developed laterally and terminally on four-celled metabasidia. Six basidiospores from two teliospores were examined and all progeny were self-sporulating. Neither self-sporulating, nor heterothallic strains of *L. antarcticum* mated with strains of other *Leucosporidium* species.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	v	Glycerol	+/w
Maltose	v	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	–
Raffinose	v	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Urease	+
Saccharate	–	Gelatin liquefaction	+
D-Glucuronate	–	Growth at 17°C	w
50% (w/w) Glucose–yeast extract agar	–	Growth at 19°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Yamada and Kondo 1972a).
Mol% G+C: 50.5 (T_m: Sugiyama et al. 1985).
Cell hydrolyzates: Not determined.

Origin of the strains studied: Antarctic marine waters at water temperatures less than 2°C in regions of pack ice adjacent to the Antarctic continent at several locations near the northern tip of the Antarctic Peninsula and at one location (150°E and 64°S) in the Indo-Pacific (46) (Fell et al. 1969, Fell 1976). The majority of the isolations were made at water depths of 100 m or less, however isolates were obtained from depths of 3000 m.

Complementary mating types: CBS 7054 (Fell strain 38-22, mating type *A1*), collected March, 1966 at 64°16′S, 150°06′E at a water depth of 152 m. CBS 7009 (Fell strain 38-107, mating type *A2*), was obtained August, 1966 from 64°10′S and 150°03′E at a water depth of 4 m.

Type strain: CBS 5942 (Fell strain AR 372), a self-sporulating strain isolated August, 1966 in the Weddell Sea near Joinville Island, Antarctica.

84.2. *Leucosporidium fellii* Giménez-Jurado & van Uden (1989)

Growth in 5% malt extract: After 3 days at 19°C, the cells are ovoidal to elongate and obpyriform, (2.7–4.0)×(8–12.7) µm; they occur singly or in pairs, and reproduction is by budding. A slight sediment is present.

Growth on 5% malt agar: After one month at 19°C, the streak culture is white to cream-colored, flat, pasty with

a fringe of true mycelium bearing complete clamps and teliospores which are predominately spherical.

Dalmau plate culture on corn meal agar: After one week at 19°C, mycelium develops with complete and incomplete clamp connections. Intercalary and terminal teliospores form that are spherical, clavate or obpyriform, (8.4–15)×(11.7–15) μm.

Life cycle: The species is homothallic; uninucleate cells develop into a dikaryotic mycelium with complete clamp connections at some, but not all of the septa. Karyogamy appears to take place in the teliospore. The teliospores germinate following 8 weeks of growth and incubation at 19°C on corn meal agar. The metabasidia are club-shaped, 3–8 μm in width and 25–45 μm in length with 2–4 cells. The basidiospores are produced on a swollen pedicel that is terminal on the distal cell and lateral on the remaining cells (Fig. 333). The basidiospores have a bacillary shape, measure (0.8–1.3)×(1.7–5.8) μm, and occur in clusters of up to six in number.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	ws	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	ws
D-Xylose	s	Succinate	w
L-Arabinose	–	Citrate	w
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

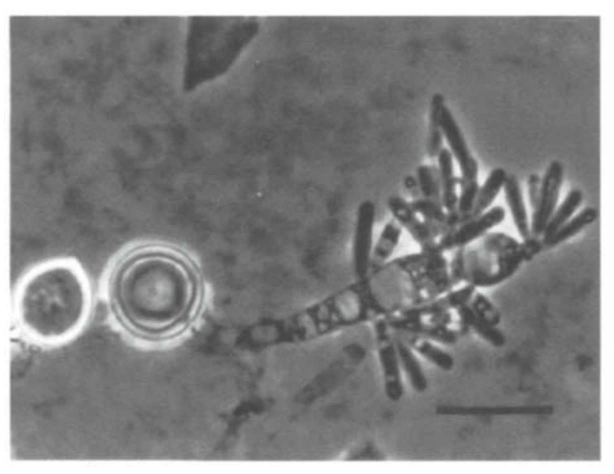


Fig. 333. *L. fellii,* CBS 7287. Cells were grown for two months on corn meal agar at 19°C; the resulting teliospore germinated to a phragmometabasidium with clusters of lateral and terminal basidiospores on swollen pedicels. Bar = 10 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Sodium nitrite	+
D-Glucuronate	+	Gelatin liquefaction	–
Arbutin	+	Glucono-δ-lactone	+
Cadaverine	+	L(+)-Tartaric acid	+
Creatine	–	Malic acid	+
Creatinine	–	0.1% Cycloheximide	+
L-Lysine	+	Growth at 19°C	+
Ethylamine hydrochloride	+	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	w		

Co-Q: 10 (Giménez-Jurado and van Uden 1989).
Mol% G + C: 57.7 (T_m: Giménez-Jurado and van Uden 1989).
Cell hydrolyzates: Not determined.

Origin of the strain examined: CBS 7287 isolated from soil collected near Oeiras, Portugal in January 1987.

Type strain: CBS 7287 (Giménez-Jurado and van Uden, I.G.C. 4403).

Comments: *L. fellii* was isolated on a selective L(+)-tartaric acid medium. L(+)-Tartaric acid is the predominant organic acid in grape must and wine and few yeasts have been found to assimilate that organic acid (Fernandez and Ruiz-Amil 1965, Giménez-Jurado and van Uden 1989). The original description reported lactose and sucrose assimilation to be negative, which differs from our results.

Giménez-Jurado and van Uden (1989) reported that the life cycle of *L. fellii* was homothallic; they presumed that the dikaryotic mycelial phase arose from the conjugation of single cells. We examined the life cycle by isolating 26 basidiospores representing progeny from seven teliospores. Based on nuclear staining (modification of Robinow 1961) the basidiospores are uninucleate and germinate to dikaryotic hyphae with complete clamp connections; the teliospores and metabasidia are uninucleate. Mating was not observed prior to teliospore formation. This type of life cycle is present in other basidiomycetous yeasts, specifically in *Sporidiobolus johnsonii, Sporidiobolus ruineniae* and *Rhodosporidium toruloides.*

According to Giménez-Jurado and van Uden (1989) "... teliospores germinate giving rise to a metabasidium which may form two or more externally located basidiospores ...". This type of basidial formation is typical of the genera *Leucosporidium, Rhodosporidium* and *Sporidiobolus,* and usually results in spheroidal to ovoid basidiospores, one to two per metabasidial cell. In contrast, we observed that *L. fellii* produced bacillary basidiospores on a swollen pedicel in whorls of six per metabasidial cell. This type of basidiospore formation is unique among the basidiomycetous yeasts although it does resemble the clusters of basidiospores depicted for *Ustilago domesticus, U. bistortarum* and *U. lagerheimii* (Fischer and Holton 1957, p. 174). This formation is probably a species-specific (rather than a

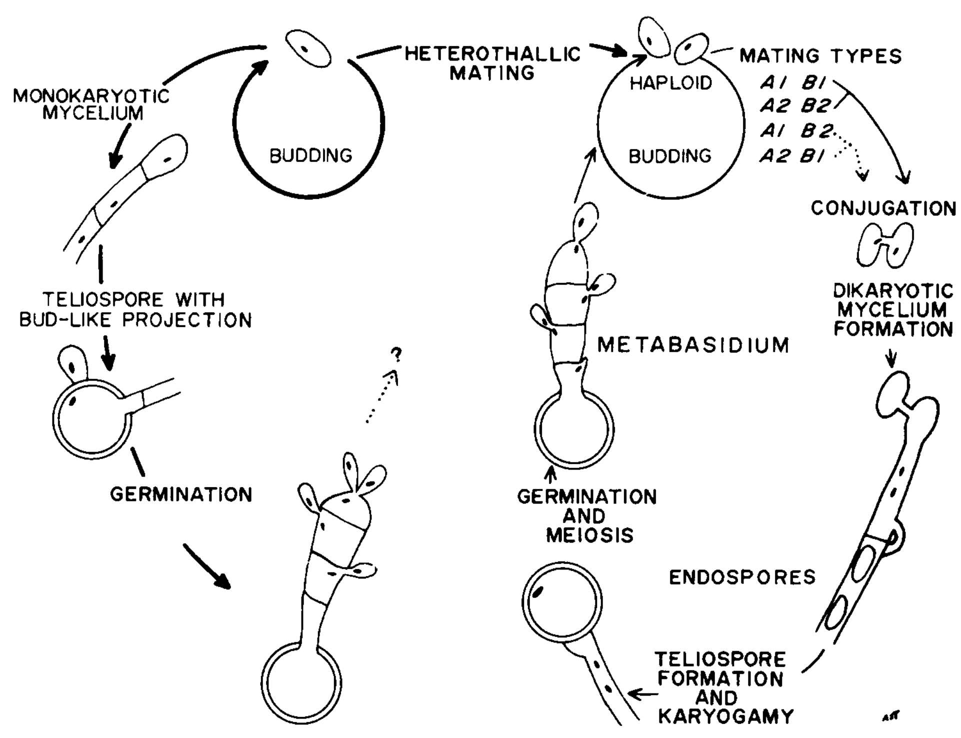


Fig. 334. *L. scottii.* Schematic drawing of the life cycle (Fell et al. 1969).

generic) characteristic among the yeasts as *L. fellii* appears to be closely related to *L. scottii* based on partial rRNA nucleotide sequence alignments (Fell and Kurtzman 1990).

84.3. *Leucosporidium scottii* Fell, Statzell, I.L. Hunter & Phaff (1969)

Anamorph: *Candida scottii* Diddens & Lodder
Synonyms:
Candida scottii Diddens & Lodder (1942)
Azymocandida scottii (Diddens & Lodder) Novák & Zsolt (1961)
Vanrija scottii (Diddens & Lodder) R.T. Moore (1980)
Apiotrichum eucryphiae Ramírez & González (1984d)

Growth in 5% malt extract: After 3 days at 25°C, cells are ovoidal to elongate, (1–7)×(4–16) µm, usually single or in pairs. Reproduction is by apical budding. Strands of pseudomycelium are present. Sediment is moderate. After one month there is a ring, some small islets and a heavy sediment.

Growth on 5% malt agar: After 3 days at 25°C, the cells are ovoidal to elongate, (1–4)×(3–12) µm. Some strands of true and pseudomycelium are evident. After one month the growth is cream-colored, extremely mucoid with dense, extensive mycelium at the periphery.

Dalmau plate culture on corn meal agar: After 3–5 days at room temperature, pseudomycelium of the *'Candida'* type begins to form. After one month, the pseudomycelium bears large clusters of blastoconidia. True mycelium with endospores is also present. Self-sporulating strains lack clamps on the teliospore-bearing hyphae.

Life cycle (Fig. 334): *L. scottii* has a multiple allelic bifactorial sexual incompatibility system. The mating competency is controlled by two factors designated *A* and *B,* which results in a minimum of four mating types: *A1B1, A2B2, A1B2, A2B1.* Present information suggests that each of the factors consists of two linked loci, designated α and β, which are separable by crossing over, i.e., a cross of $A\alpha_1\beta_1$ strain with $A\alpha_2\beta_2$ strain can result in recombinant progeny $A\alpha_1\beta_2$ and $A\alpha_2\beta_1$. Five *A* and three *B* factors have been identified (Fell and Statzell-Tallman 1982). When compatible strains are mixed on corn meal agar at 25°C, they conjugate within 30 hours at a frequency of less than 1%. A binucleate hypha develops from one of the conjugate cells.

After 72 hours, the hyphae develop extensively with clamp connections at each septum. After 4–7 days intercalary and terminal teliospores develop with one or more clamp structures between the spore and hypha. The teliospores are spherical, thick-walled, have a granular content and range in size from (7–15)×(7–16) µm. Karyogamy in the teliospores precedes germination. After 5 days, the teliospore (Fig. 335) germinates and the diploid nucleus migrates into the metabasidium. Meiosis occurs in the metabasidium, which partitions into four cells. Each metabasidial cell contains one nucleus, which reproduces mitotically, resulting in one nucleus in the cell and one in the developing basidiospore. Basidiospores form near the septa and terminally on the distal metabasidial cell. Budding of the basidiospores results in mucoid yeast cells that represent either parental mating types or self-sporulating strains.

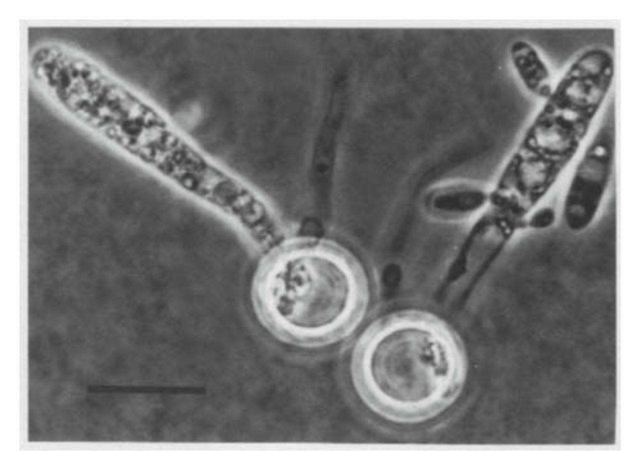


Fig. 335. *L. scottii*, CBS 5930×CBS 5931. Cells of opposite mating types were mixed on corn meal agar and incubated at 25°C for 5 days; the resulting teliospores germinated to a four-celled metabasidium with lateral basidiospores. Bar = 10 μm.

Some strains are capable of monokaryotic formation of teliospores: a hypha with uninucleate cells develops from a uninucleate yeast cell. The hyphae do not have clamp connections, although incomplete clamp-like structures may be present. Monokaryotic fruiters will mate with heterothallic mating strains resulting in binucleate clamped hyphae with teliospores. Progeny of matings of monokaryotic×heterothallic strains are 50% heterothallic fruiters and 50% monokaryotic fruiters. Similar results have been obtained with heterothallic×heterothallic fruiters. This segregation suggests that there are one or more separate genes for fruiting that may be repressed in some heterothallic strains. This gene may not be associated with a specific mating type as suggested by the mating of monokaryotic *A3B1*×heterothallic *A2B2* → heterothallic *A3B1* progeny (Fell and Statzell-Tallman 1982). Strains of *L. scottii* do not mate with strains of other species in the genus.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	w	Ribitol	v
Trehalose	+	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	v	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Urease	+
Saccharate	v	Gelatin liquefaction	+
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 9 and 10 (Yamada and Matsumoto 1988a); 8, 9 and 10 as minor subunits (Yamada and Nakagawa 1990); 9 (CBS 5931) and 10 (CBS 614) (Sugiyama et al. 1985, Yamada and Kondo 1972a).
Mol% G + C: 60.9, 61.1 (T_m: Giménez-Jurado and van Uden 1989).
Cell hydrolyzates: Glucose, mannose and galactose (Gorin and Spencer 1970).

Origin of the strains studied: 65 strains were examined: Southern Pacific Ocean (59) (Fell 1976); Antarctic soil and seawater strains from di Menna (3); soil in Australia (1) (Lodder and Kreger-van Rij 1952); unreported sources (2).

Complementary mating types: CBS 6562 (*A1B2*) and CBS 6561 (*A2B1*) are progeny from *A1B1*×*A2B2* matings (Fell 1974). CBS 5932 (a self-sporulating strain) was collected August 1966 at 134°53′W, 34°59′S from Antarctic seawater at a water depth of 155 m, temperature of 2.59°C and salinity of 34.61‰.

Type strain: CBS 5930 (mating type *A2B2*) collected August 1966 from Antarctic seawater at 129°59′W, 41°58′S at a water depth of 1364 m, temperature of 3.0°C, salinity of 34.44‰.

Allotype: CBS 5931 (mating type *A1B1*) was collected August 1966 from Antarctic seawater (134°30′W, 36°00′S) at a water depth of 1902 m, temperature of 2.3°C and salinity of 34.62‰.

Comments: *L. scottii* inhabits low temperature environments, generally in polar regions or in temperate regions during the cold weather seasons. There have been no reports from tropical or sub-tropical environments. Summerbell (1983) reviewed the ecology of *L. scottii* and recorded the various isolation sources. These included soil and frozen beef from Australia (Scott 1936); apple fruit in Canada (Clark et al. 1954), soil in New Zealand (di Menna 1955a,b, 1958a, 1960, 1965a); grapevines in South Africa (Van Zyl and du Plessis 1961); plant blossoms in Czechoslovakia (Kocková-Kratochvílová 1964); wild mushroom in Czechoslovakia (Kocková-Kratochvílová et al. 1965); soil and grapes in New Zealand (Parle and di Menna 1966); polluted rivers in the northern United States (Woollett and Hedrick 1970), Quebec (Simard and Blackwood 1971) and France (Hinzelin and Lectard 1978); marine fish in Scotland (Bruce and Morris 1973); Antarctic soils (di Menna 1960, 1966b); Antarctic seas (Fell et al. 1969, Fell 1974); Antarctic mosses and saline lakes (Goto et al. 1969); soils in East Greenland (di Menna 1966b); Soviet Arctic tundra soils (Bab'eva et al. 1976, Bab'eva and Azieva 1980). Summerbell (1983) isolated *L. scottii* in southern British Columbia,

Canada from snow and decomposing plant materials in terrestrial locations, foam, living and decomposing plant material in fresh water and plants and driftwood in marine locales.

Yamada and Nakagawa (1990) examined the coenzyme Q systems and partial nucleotide sequences of the SSU and LSU rRNA of 19 strains of *L. scottii*. They found one strain IFO 9474 (UBC 670 from Bandoni) with Co-Q 7 and a sequence which differed from the other *L. scottii* strains – apparently this strain is a distinct species from *L. scottii*. The remaining strains were either Co-Q 9 or Co-Q 10. Eight strains had Co-Q 9 as the major component; three of these strains were isolated by Fell et al. (1969) from Antarctic sea water, the remaining 5 strains were progeny isolated from conjugants that included those marine strains (Fell and Statzell-Tallman 1982). The Co-Q 10 containing strains were isolated either from terrestrial sources or from temperate marine regions. Nucleotide sequence alignments of rRNA from Co-Q 9 and Co-Q 10 containing strains indicated a single species (Yamada and Nakagawa 1990, Fell and Kurtzman 1990).

Comments on the genus

Based on septal morphology, polysaccharide composition of the cell hydrolyzates and molecular phylogeny (see Moore, Chapter 5, Kurtzman and Blanz, Chapter 10, and Boekhout et al., Chapter 74, this volume), *Leucosporidium* is a teliospore-forming member of the Sporidiales. Three species are included in the genus, all have white to cream-colored colonies and appear to have low optimal growth temperatures. Two of the species (*L. antarcticum* and *L. scottii*) have been isolated from polar and other cold temperature regions. The species differ in their methods of teliospore formation: self-sporulation, homothallic uni- and bi-allelic systems; however in all cases a phragmometabasidium with basidiospores is formed. *L. fellii* produces distinctive basidiospores: bacillary basidiospores on a swollen pedicel in whorls of six per metabasidial cell (see Fig. 333). The *Leucosporidium* species can be separated from each other and from other teliospore-forming yeasts by standard fermentation and assimilation tests (Table 69).

85. *Mrakia* Y. Yamada & Komagata

J.W. Fell and A. Statzell-Tallman

Diagnosis of the genus

Growth on solid media is white to cream-colored, often mucoid. Visible carotenoid pigments are not present. Both yeast and mycelial phases occur. In the yeast phase, the cells are ovoid to elongate and reproduce by budding; a pseudomycelium may form. Formation of the mycelial phase occurs in the absence of mating. Teliospores are produced terminally or intercalarily on the mycelium. Yeast cells are uninucleate and produce uninucleate teliospore-bearing hyphae without clamp connections. Teliospore germination results in phragmo- or holometabasidia. Ballistoconidia are not present in either the yeast or mycelial phase. The septal pore is a dolipore without a parenthesome.

Glucose is fermented. Nitrate is assimilated. *Myo*-inositol is not assimilated. Maximum temperature for growth is below 20°C. Xylose is present in cell hydrolyzates. Starch-like compounds are produced. Diazonium blue B and urease reactions are positive. Coenzyme Q-8 system is present.

Type species

Mrakia frigida (Fell, Statzell, I.L. Hunter & Phaff) Y. Yamada & Komagata

Species accepted

1. *Mrakia frigida* (Fell, Statzell, I.L. Hunter & Phaff) Y. Yamada & Komagata (1987)

Systematic discussion of the species

85.1. *Mrakia frigida* (Fell, Statzell, I.L. Hunter & Phaff) Y. Yamada & Komagata (1987)

Synonyms:
Leucosporidium frigidum Fell, Statzell, I.L. Hunter & Phaff (1969)
Candida frigida di Menna (1966a)
Vanrija frigida (di Menna) R.T. Moore (1980)
Leucosporidium gelidum Fell, Statzell, I.L. Hunter & Phaff (1969)
Mrakia gelida (Fell, Statzell, I.L. Hunter & Phaff) Y. Yamada & Komagata (1987)
Candida gelida di Menna (1966a)
Vanrija gelida (di Menna) R.T. Moore (1980)
Leucosporidium nivalis Fell, Statzell, I.L. Hunter & Phaff (1969)
Mrakia nivalis (Fell, Statzell, I.L. Hunter & Phaff) Y. Yamada & Komagata (1987)
Candida nivalis di Menna (1966a)
Vanrija nivalis (di Menna) R.T. Moore (1980)
Leucosporidium stokesii Fell, Statzell, I.L. Hunter & Phaff (1969)
Mrakia stokesii (Fell, Statzell, I.L. Hunter & Phaff) Y. Yamada & Komagata (1987)
Candida curiosa Komagata & Nakase (1965)
Cryptococcus curiosus (Komagata & Nakase) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Growth in 5% malt extract: After 3 days at 12°C, the cells are short-ovoidal to ovoidal-elongate, (2.6–8.0)×(3–14) μm, and occur singly or in pairs. Occasionally septate cells are observed. A sediment is formed and sometimes a slight ring. After one month, a ring, which may be thick and myceliated, and a light to heavy sediment may be present.

Growth on 5% malt agar: After one month at 12°C, the streak culture is cream-, yellowish-white to light brownish-gray, raised, dull, mucoid and smooth or wrinkled. The margin may be fringed with true mycelium bearing ovoid to ovoid-elongate teliospores but no clamp connections. The teliospores may be intercalary or terminal and spherical (5–8 μm in diameter) or elongate (5.4–11.9)×(6.5–13.5) μm.

Dalmau plate culture on corn meal agar: After one week, pseudo- and true mycelium are abundantly developed. Blastoconidia are short-ovoid to ovoid and occur singly, in chains, in clusters or in verticils. Teliospores form occasionally or abundantly, usually in greater numbers after 3 weeks when they are smaller in size, (3–7)×(4–8) μm, as compared to their larger size at 9 weeks, (4–11)×(5–16) μm.

Life cycle: Mycelium and teliospores develop directly from a single cell and no clamp connections form. Nuclear stains indicate that the mycelium is uninucleate. Teliospores may germinate to a one to three celled metabasidium, 8–12 μm long (Fig. 336). Germination has not been observed in all strains. Teliospores of certain strains germinate in response to different regimes of varied temperatures (5°C–12°C) and time of growth on corn meal agar or 2% glucose Sabouraud agar, soaking in distilled water and streaking on 2% agar devoid of added nutrients. Basidiospores are terminal and/or lateral (Fig. 336).

Fermentation:

Glucose	s	Trehalose	–
Galactose	v	Melezitose	–
Sucrose	v	Inulin	–
Maltose	v	Melibiose	–
Lactose	–	Cellobiose	–
Raffinose	v		



Fig. 336. *M. frigida*, NRRL Y-7203. After 5 weeks on corn meal agar at 12°C followed by 7 weeks in distilled water, teliospore germination with terminal basidiospores on a one-celled metabasidium occurred on 2% agar after 2 weeks. Bar = 10 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	s	Ethanol	s
Sucrose	+	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	s	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	–
D-Xylose	s	Succinate	v
L-Arabinose	+	Citrate	v
D-Arabinose	v	Inositol	w/–
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	+	Gelatin liquefaction	v
Saccharate	v	Biotin-free	–
D-Glucuronate	s	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 17°C	+/w
		Growth at 19°C	v
10% NaCl/5% glucose	–	Growth at 25°C	–
Starch formation	+		

Co-Q: 8 (Yamada and Kondo 1972a).

Mol% G + C: 54.1, *M. frigida* (T_m: Nakase and Komagata 1971f); 56.1, *M. gelida* (T_m: Nakase and Komagata 1971f); 52.9, CBS 5688, *Candida curiosa* (T_m: Meyer et al. 1984).

Cell hydrolyzates: Xylose present (Sugiyama et al. 1985).

Origin of the strains studied: Di Menna (1966b) obtained approximately 80 isolates from Antarctic soil samples collected in the vicinity of Campbell–Mawson, Mawson–Koettlitz, Beardmore–Nimrod, Shackelton–Axel Heiberg Glaciers and from Scott Base. She also obtained 20 isolates from Greenland soil samples near Mastersvig airstrip. The common constituent of the Greenland samples was plant material such as mosses, lichens and algae. Di Menna's strains, used as type strains in the description of *Leucosporidium* spp., were: 5A1 (CBS 5270) *L. frigidum*; 2AH1O (CBS 5272) *L. gelidum*; 2AH2 (CBS 5266) *L. nivalis*. Sinclair and Stokes (1965) isolated a strain (P-16, CBS 5917), from an Antarctic snow core, which was described as *L. stokesii.* Komagata and Nakase (1965) isolated two strains, P-6 (CBS 5688) and P-13, from frozen food (*Sillago japonica*) in Kawasaki, Japan, 1963, which the authors described as *Candida curiosa.* M. Parish (Univ. Florida) obtained a strain from orange juice in cold storage (5°C) in Florida.

Type strain: CBS 5270, isolated from soil at Scott Base, Antarctica, by M. di Menna.

Comments on the genus

Di Menna (1966a,b) isolated a group of low temperature yeasts from Antarctic and Greenland soil samples and described several species of *Candida*: *C. frigida, C. gelida* and *C. nivalis* based on differences in assimilation patterns. These species were placed in the genus *Leucosporidium* due to the observation of teliospore formation, which was a result of self-sporulation. The species were retained as separate *Leucosporidium* species, based on physiological properties as per di Menna's recommendations. Yamada and Komagata (1987) studied the Co-Q system of the genus *Leucosporidium* and found that the original di Menna species, and *L. stokesii,* differed from other members of *Leucosporidium,* by the presence of the Co-Q 8 system. Sugiyama et al. (1985) reported the presence of xylose in the cell hydrolyzates of these species; in contrast, xylose is absent in other species of *Leucosporidium.* Based on these differences Yamada and Komagata (1987) created the genus *Mrakia* consisting of four species that they transferred from *Leucosporidium.* Yamada and Matsumoto (1988a) examined the electrophoretic pattern of 7 enzymes of strains of *Mrakia* spp. and concluded that *M. nivalis* was a synonym of *M. frigida* and that *M. stokesii* was a synonym of *M. gelida.* Molecular sequence analysis (Yamada and Kawasaki 1989a, Fell et al. 1992) suggests that all four nomenclatural strains represented a single species, *M. frigida.*

We also include *Candida curiosa* in the list of synonyms. Van Uden and Buckley (1970) recognized the synonymy of *C. curiosa* and *C. nivalis* based on physiological properties. Because teliospores had not been observed in *C. curiosa,* the species was retained in *Candida* (Meyer et al. 1984) and subsequently transferred to *Cryptococcus* as *C. curiosus* (Weijman et al. 1988). During the examination of *C. curiosus* for the present treatise, we were able to induce teliospore formation by incubating a culture on 2% dextrose Sabouraud agar at 12°C to confirm the synonymy recommended by van Uden and Buckley (1970).

86. *Rhodosporidium* Banno

J.W. Fell and A. Statzell-Tallman

Diagnosis of the genus

Colony color on solid media includes shades of yellow, orange and red due to the presence of carotenoid pigments. Yeast and mycelial phases may occur. In the yeast phase the cells are ovoid to elongate and reproduce by budding; a pseudomycelium may form. Formation of the mycelial phase can result from sexual reproduction and may develop by two methods of self-sporulation. True mycelium with or without clamp connections is formed. The septal structure consists of a simple pore. Teliospores may be terminal, lateral on stalks or intercalary on the mycelium.

Sexual mechanisms, although not completely understood, are both homothallic and heterothallic. Heterothallism includes unifactorial biallelic (e.g., *R. toruloides*) and bifactorial (*R. dacryoideum*) systems. In these heterothallic systems a dikaryotic mycelium results from the conjugation of a compatible mating pair. A clamp connection is usually present at each cross wall. Intercalary and terminal teliospores develop. Karyogamy takes place in the teliospore (probasidium), which germinates to produce a two to four celled, septate metabasidium (phragmometabasidium). Meiosis takes place during germination and the resulting haploid basidiospores are lateral and terminal on the phragmometabasidium.

Homothallism, or self-fertility, consists of the development of hyphae with teliospores from a single yeast cell in the apparent absence of mating. The yeast phase is diploid and reduction division takes place with the formation of a dikaryotic mycelium with clamp connections. Karyogamy occurs in the teliospore. Germination of the teliospore results in a metabasidium with diploid basidiospores (strains of *R. dacryoideum* and *R. toruloides*).

In contrast to the homothallic life cycle, the hyphal cells of some self-sporulating species (e.g., *R. fluviale* and *R. kratochvilovae*) remain uninucleate and clamp connections are lacking. Teliospore formation has not been studied in detail, although the process may be monokaryotic fruiting rather than a sexual cycle. Neither ballistospores nor ballistoconidia are present in any of the phases.

Sugars are not fermented. Diazonium blue B and urease reactions are positive. Extracellular starch is not produced. Among the species currently included in the genus, none assimilate lactose, melibiose, D-glucosamine, erythritol, or inositol. Galactose, trehalose and glycerol are assimilated. Xylose is absent in the cells. Coenzymes Q-9 and Q-10 are present.

Type species

Rhodosporidium toruloides Banno

Species accepted

1. *Rhodosporidium babjevae* Golubev (1993)
2. *Rhodosporidium dacryoideum* Fell, I.L. Hunter & Tallman (1973)
3. *Rhodosporidium diobovatum* Newell & I.L. Hunter (1970)
4. *Rhodosporidium fluviale* Fell, Kurtzman, Tallman & Buck (1988)
5. *Rhodosporidium kratochvilovae* Hamamoto, Sugiyama & Komagata (1988)
6. *Rhodosporidium lusitaniae* A. Fonseca & Sampaio (1992)
7. *Rhodosporidium malvinellum* Fell & I.L. Hunter (1970)
8. *Rhodosporidium paludigenum* Fell & Statzell-Tallman (1980)
9. *Rhodosporidium sphaerocarpum* Newell & Fell (1970)
10. *Rhodosporidium toruloides* Banno (1967)

Key to species

See Table 70.

1.	a Ballistoconidia formed	*Sporidiobolus* spp:	p. 693
	b Ballistoconidia not formed → 2		
2(1).	a Inositol utilized, extracellular starch produced	*Cystofilobasidium* spp:	p. 646
	b Inositol not utilized, extracellular starch not produced → 3		
3(2).	a Teliospores formed by mating → 4		
	b Teliospores not formed by mating → 10		
4(3).	a Teliospores markedly angular	*R. toruloides*:	p. 690
	b Teliospores not markedly angular → 5		
5(4).	a Nitrate assimilated → 6		
	b Nitrate not assimilated	*R. dacryoideum*:	p. 680
6(5).	a Melezitose assimilated → 7		
	b Melezitose not assimilated → 9		

7(6). a Teliospores diobovate or diclavate .... *R. diobovatum*: p. 682
b Teliospores globose to subglobose → 8
8(7). a L-Arabinose assimilated .... *R. sphaerocarpum*: p. 688
b L-Arabinose not assimilated .... *R. babjevae*: p. 679
9(6). a Colony color salmon-orange .... *R. paludigenum*: p. 687
b Colony color white to mauve .... *R. malvinellum*: p. 686
10(3). a Teliospores highly angular .... *R. toruloides*: p. 690
b Teliospores not highly angular → 11
11(10). a Nitrate assimilated → 12
b Nitrate not assimilated .... *R. dacryoideum*: p. 680
12(11). a Melezitose assimilated → 13
b Melezitose not assimilated .... *R. lusitaniae*: p. 685
13(12). a Growth in vitamin-free medium → 14
b Growth absent in vitamin-free medium .... *R. sphaerocarpum*: p. 688
14(13). a Galactitol assimilated .... *R. fluviale*: p. 683
b Galactitol not assimilated .... *R. kratochvilovae*: p. 684

Table 70
Key characters of species in the genus *Rhodosporidium*

Species	Teliospore formation			Colony color	Assimilation[c]				Growth in vitamin-free medium
	HM or SS[a]	Heterothallic	Shape[b]		Mz	L-Ar	Gal	NO_3	
Rhodosporidium babjevae	–	+	glo	red	+	–	–	+	s
R. dacryoideum	SS	+	dac	red-orange	v	–	–	–	–
R. diobovatum	–	+	dio	coral-red	+	+	v	+	+
R. fluviale	SS	–	glo	scarlet-orange	+	+	+	+	+
R. kratochvilovae	SS	–	glo	peach-orange	+	+	–	+	+
R. lusitaniae	HM	–	glo	peach	–	s	–	+	+
R. malvinellum	–	+	sub	white to mauve	–	v	v	+	–
R. paludigenum	–	+	glo	salmon-orange	–	s	+	+	+
R. sphaerocarpum	SS	+	glo	orange	+	+	–	+	–
R. toruloides	HM	+	ang	scarlet-orange	+	+	–	+	+

[a] Abbreviations: HM, homothallic; SS, self-sporulating formation of teliospores.
[b] Abbreviations: glo, globose; dac, dacryoid; dio, diobovate; ang, angular.
[c] Abbreviations: Mz, melezitose; L-Ar, L-arabinose; Gal, galactitol; NO_3, nitrate.

Systematic discussion of the species

86.1. *Rhodosporidium babjevae* Golubev (1993)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are spheroidal to ovoidal (the width/length ratios are from 1.0 to 2.0), (2.5–6.0)×(4.2–8.5) µm, with small capsules, and single or in pairs. After one month, a sediment, a ring and also islets or a pellicle sometimes form.

Growth on yeast morphology agar: After one month, the streak cultures are red, smooth, glistening, mucoid or soft; the margin is entire to undulating.

Slide cultures on corn meal agar: After 10 days, neither pseudomycelium nor true mycelium is produced. Short lengths of true hyphae without clamp connections may be present on month-old cultures. No ballistoconidia are observed.

Life cycle: Conjugation of mating types on corn meal agar at 20°C, results in the formation of true mycelium with clamp connections at the septa. Two mating types were found (Golubev 1993, Bab'eva and Kartintsev 1974), which suggests a unifactorial mating system. The teliospores are subglobose, globose (6.0–12.8)×(7.6–13.6) µm, usually single, terminal, thick-walled, and granular. After soaking 2 week-old cultures in distilled water for 3 days, teliospores germinate to produce 1 or 2-celled metabasidia with 1 to 3 terminal ovoid basidiospores (Figs. 337, 338). *R. babjevae* does not mate with other species included in the genus *Rhodosporidium,* or with the type strain of *Rhodotorula glutinis.*

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	s	Ethanol	s
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+/w	Ribitol	w/–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	s
Inulin	w/–	D-Gluconate	n
Soluble starch	w	DL-Lactate	s
D-Xylose	w/–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	s	Inositol	–
D-Ribose	ws	Hexadecane	n
L-Rhamnose	s	Nitrate	+
D-Glucosamine	–	Vitamin-free	s

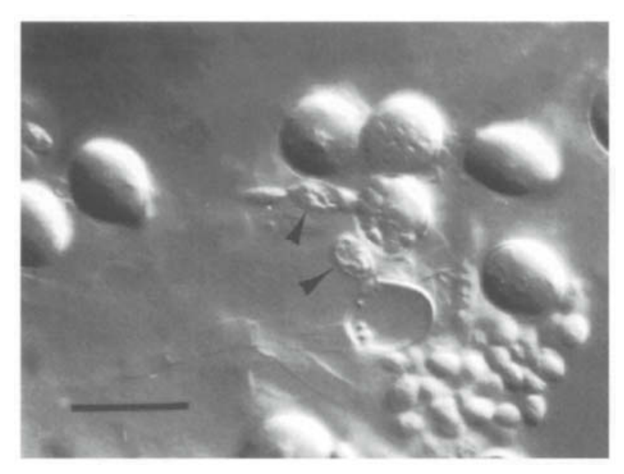


Fig. 337. *R. babjevae,* CBS 7808×CBS 7809. Teliospores, which developed from a mix of opposite mating type cells after two weeks on corn meal agar at 25°C, were soaked for 3 days in deionized water; germination occurred after 2 days on 2% agar. Arrows indicate metabasidia; the upper metabasidium has a terminal basidiospore attached. Bar = 10 µm.

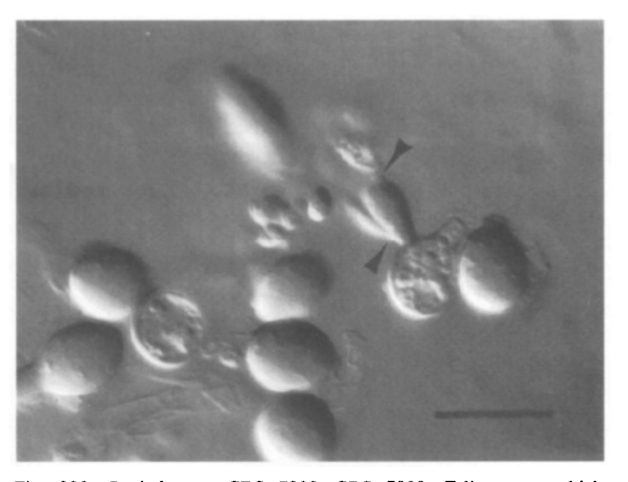


Fig. 338. *R. babjevae,* CBS 7808×CBS 7809. Teliospores, which developed from a mix of opposite mating type cells after two weeks on corn meal agar at 25°C, were soaked for 3 days in deionized water; germination occurred after 2 days on 2% agar. The elongate basidiospore from the basal cell is on a short pedicel (indicated by lower arrow), and typically grows parallel to the metabasidium. The terminal cell has a pedicel (indicated by upper arrow); the nearby cell is not attached to the pedicel. Bar = 10 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	–	50% Glucose–yeast extract agar	s
Saccharate	n		
D-Glucuronate	–	10% NaCl/5% glucose	n
Xylitol	w	Starch formation	–
L-Arabinitol	–	Urease	+
Arbutin	+	Nitrite	s
Cadaverine	s	Gelatin liquefaction	n
Creatine	–	D-Glucono-δ-lactone	+
Creatinine	–	Growth at 30°C	+
L-Lysine	s	Growth at 37°C	–

Co-Q: 10 (Golubev 1993).
Mol% G + C: 66.2 (PC: Golubev 1993).
Monosaccharide composition of extracellular polysaccharides: Mannose, galactose, glucose, and fucose (Golubev 1993).

Origin of the strains studied: Herbaceous plants, Moscow Region, Russia, from Bab'eva et al. (1973).

Complementary mating types: CBS 7808 (VKM Y-2275, mating type *A1*) and CBS 7809 (VKM Y-2276, mating type *A2*).

Type strain: CBS 7808.

Comments: This species came to our attention after the completion of this generic review, therefore the data recorded above are from the original description. We briefly examined teliospore germination from mixes of compatible mating types on corn meal agar for 2 weeks at 25°C. After soaking the teliospores in sterile deionized water for one week and moving them to 2% agar, germination occurred and 1–2 celled metabasidia (6 µm×2.6 µm) form (Fig. 337). More measurements of metabasidia are required to assure an accurate assessment of size. The basidiospores formed on small persistent pedicels and up to 3 pedicels were observed at different sites on the terminal cell of 2-celled metabasidia. We also observed single basidiospores on pedicels from the basal cell. These basidiospores may grow parallel to the basal cell (Fig. 338). Additional strains were collected from soil, Krasnodar Region, Russia, by Golubev and Vdovina (1973) (2); silage, Kiev Region, Ukraine, by Shchelokova (1964) (1) and from peat, birch leaves, soils in Novgorod, Nizhni Novgorod regions and East Pamir by Bab'eva and Kartintsev (1974).

86.2. *Rhodosporidium dacryoideum* Fell, I.L. Hunter & Tallman (1973)

Synonym:
Sakaguchia dacryoides (Fell, I.L. Hunter & Tallman) Y. Yamada, Maeda & Mikata (1994a)

Growth in 5% malt extract: After 3 days at 24°C, the haplophase cells are ovoid, (1–5)×(3–9) µm, single, in pairs and short chains; budding may occur on a short stalk. A ring and film are not present; there is a light sediment. After one month, there is a light to heavy ring and moderate sediment.

Growth on 5% malt agar: After 7 days at 24°C, the growth is red-orange, smooth, creamy, glistening, and slightly raised; the margin is entire. Cell morphology is similar to that in malt extract. After one month, the growth is slightly darker in color, glistening, creamy to pasty; occasionally the edge is slightly wrinkled.

Dalmau plate culture on corn meal agar: After one week at 24°C, mycelial growth may be absent or there may be long thin true mycelium or short primitive pseudomycelium.

Life cycle (Fig. 339): The species is heterothallic (bifactorial), although a self-sporulating strain has been observed. Mating pairs with dissimilar *A* and *B* factors (*A1B1*×*A2B2*, *A1B2*×*A2B1*) produce the complete life cycle. Mixing of strains with similar *A* and dissimilar *B* factors results in mating and formation of short,

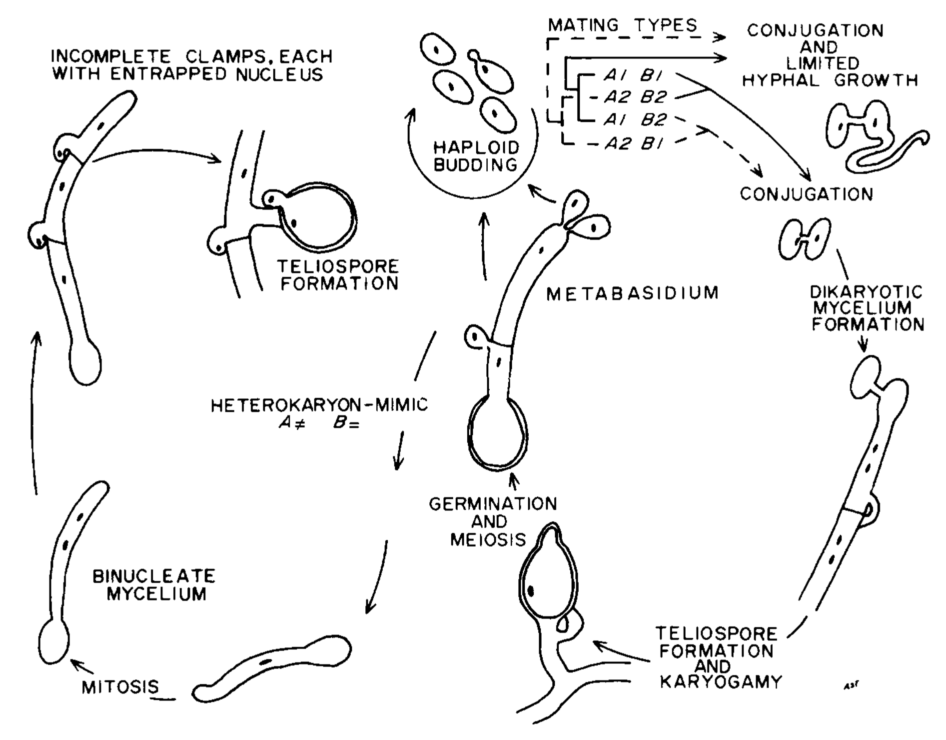


Fig. 339. Life cycle *R. dacryoideum*, CBS 6353×CBS 7142 (Fell et al. 1973).

distorted, true hyphae lacking both clamp connections and teliospores. There is no mating reaction with pairs having similar *B* factors. Conjugation between mating types with opposite *A* and *B* factors is usually within 24 hours of mixing on corn meal agar at 24°C; this may be variable, mating may not occur for one week or longer. After one week binucleate mycelium is sparse to abundant with hook-to-peg clamp connections. Conidia have not been observed on the hyphae. Teliospores are lateral or occasionally terminal on the hyphae. The lateral teliospores develop either directly from the hypha on a short sporophore or from clamp connections. After one week the teliospores are subglobose and slightly dacryoid (4–10)×(5–14) μm, or more pronounced dacryoid with a subglobose base (4–8)×(7–10) μm and an apical tip measuring (0.3–3)×(0.8–6) μm. After one month most teliospores exceed 6×11 μm. The apical tip may be wider than long (1–2)×(2–3) μm. Basal clamp connections join the teliospore to the hypha or to the sporophore. Germination of the teliospores is often difficult to obtain. The best results were obtained by soaking one month old cultures in distilled water at 24°C. After 10–25 days of soaking, germination (Fig. 340) was observed directly from the distilled water preparation. The metabasidium was 2 to 4-celled, (3–4)×(24–59) μm with lateral and terminal basidiospores.

Progeny were isolated by micromanipulation of basidiospores derived from *A1B1*×*A2B2*. Resulting F_1 mating types were *A1B1, A2B2, A2B1,* and one strain similar to the *A*≠*B*= heterokaryons of *Coprinus lagopus* and *Schizophyllum commune* (see Raper 1966, for a discussion of this behavior). The yeast cells of the *A*≠*B*= strain were generally uninucleate, although binucleate cells were present. A mycelium developed that was initially binucleate; during the process of clamp formation and nuclear segregation, the clamps were not completed and one nucleus remained in the clamp and one nucleus was in the subterminal cell. The terminal cells were uninucleate. Teliospores were formed. Conjugation was not observed when the individual mating types were mixed with strains of the other species in the genus.

Fermentation: absent.



Fig. 340. *R. dacryoideum*, CBS 6353×CBS 7142. A teliospore, which developed from a mix of opposite mating type cells after 4 weeks on corn meal agar, was soaked for 2 weeks at 24°C; it germinated to a 2-celled metabasidium with lateral basidiospores. Bar = 10 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	w/–
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	v	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+/w
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	s
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Urease	+
5-Keto-D-gluconate	n	Gelatin liquefaction	–
Saccharate	–	PABA-free	w
D-Glucuronate	s	Thiamine-free	w
50% Glucose–yeast extract agar	–	Growth at 30°C	+
10% NaCl/5% glucose	–	Growth at 33°C	–
Starch formation	–		

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G+C: 57.8, 58.1, 58.4, 58.9 (T_m: Hamamoto et al. 1986b).
Cell hydrolyzates: Xylose absent (Sugiyama et al. 1985).

Origin of the strains studied: Marine waters of the southern Indian and Pacific Oceans (10) in surface waters to depths of 4156 m at water temperatures from 0.6°C to 8.0°C (Fell et al. 1973).

Complementary mating types: CBS 6353 (mating type *A1B1*) was collected April 1966, at 61°31′S latitude, 95°56′W longitude, at a water depth of 1738 m, temperature of 1.8°C and salinity of 34.72‰; CBS 6354 (mating type *A2B2*) collected July 1966, 43°00′S, 139°59′W, 156 m deep, 80°C and 34.40‰; CBS 6355 (mating type *A2B1*) collected April 1966, 61°31′S, 95°56′W, 3929 m deep, 0.61°C, 34.71‰; CBS 6356 (mating type *A1B2*) collected April 1966, 61°31′S, 95°56′W, 3 m deep, 4.02°C and 33.96‰ (Fell et al. 1973). CBS 6357 is an $A \neq B =$ strain from parental strains CBS 6353 and CBS 6354.

Type strain: CBS 6353.

Comments: The species is widespread in Antarctic marine waters; we are not aware of any other sources of isolates. Haploid strains of *R. dacryoideum* fit the description of *Rhodotorula minuta,* however the type strain of *Rhodotorula minuta* does not mate with the mating types of *R. dacryoideum.* Nucleotide sequence analysis of a partial region of the LSU rRNA also indicates that they are separate species (Fell et al. 1992).

86.3. *Rhodosporidium diobovatum* Newell & I.L. Hunter (1970)

Synonym:
?*Torulopsis terrestris* Verona (1935)

Growth in malt extract: After 3 days at 22°C, the haplophase cells are round to ovoid (1–6)×(2–9) μm, and occur singly or in pairs. A slight sediment is formed. After one month, there is a heavy sediment and a thin to moderate film of cells; large round cells (to 9–11 μm diameter) with large oil globules are present.

Growth on malt agar: Cell morphology is similar to that in malt extract. The streak colony is coral-red, raised, mucoid, the border entire.

Dalmau plate culture on corn meal agar: Some strains produce only budding cells, whereas other strains produce, in addition to budding cells, a primitive pseudomycelium or fine, true hyphae (diameter 2 μm) bearing blastoconidia with dimensions similar to the yeast-phase cells. These blastoconidia are often formed at septa, on structures homologous to clamp connections, but aberrant and incomplete. Each primary hypha is formed by a single yeast-phase cell.

Life cycle: The species is heterothallic; a biallelic (*A1* and *A2*) system of sexual incompatibility factors exists. Mixing of yeast-phase strains of *A1* and *A2* on corn meal agar results in conjugation within 24 hours. Plasmogamy takes place, and binucleate hyphae (diameter 3 μm) are formed from one of the conjugant cells, or from the conjugation tube joining them. Clamp connections are hook-to-peg or peg-to-peg. After 3 days of hyphal growth, unicellular, round to obovate, diobovate, or diclavate, (6–11)×(6–22) μm, heavy-walled terminal teliospores form. The teliospores have clamp connections at the basal septum and are binucleate at first, but become uninucleate at maturity. In addition to teliospores, blastoconidia form on the binucleate hyphae. Additional incompatibility factors may exist as several isolates have been examined

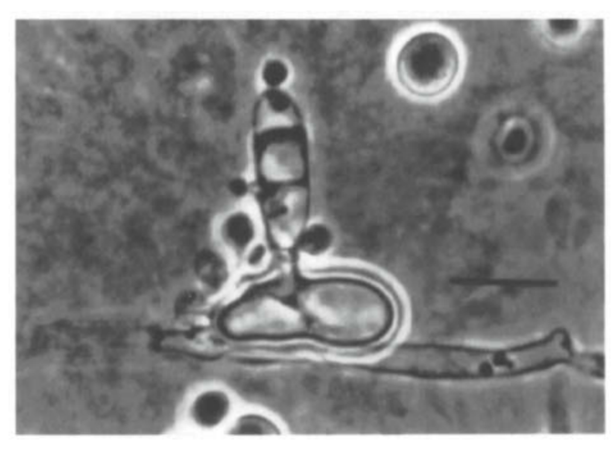


Fig. 341. *R. diobovatum,* CBS 6084×CBS 6085. Teliospore, which developed from a mix of opposite mating type cells after 5 days on corn meal agar at 22°C, developed a lateral metabasidium with lateral and terminal basidiospores, within 10 days of soaking in distilled water. Bar = 10 μm.

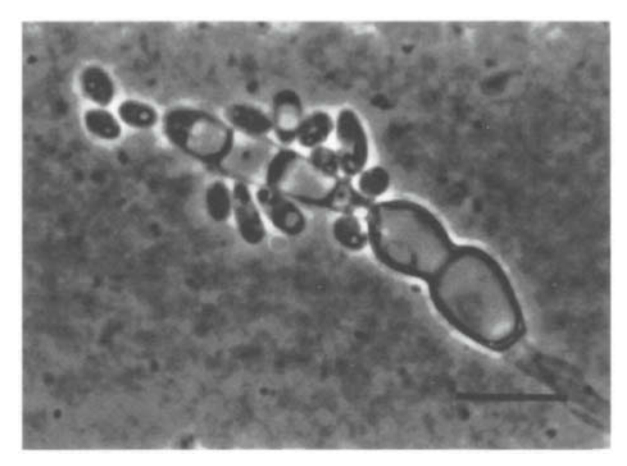


Fig. 342. *R. diobovatum,* CBS 6084×CBS 6085. Teliospore, which developed from a mix of opposite mating type cells after 5 days on corn meal agar at 22°C, developed a terminal metabasidium with lateral and terminal basidiospores, within 10 days of soaking in distilled water. Bar = 10 µm.

which mate with *A2* strains to produce mycelium but fail to develop teliospores. Germination of the teliospores occurs after 5–10 days of soaking in distilled water. A lateral (Fig. 341) or terminal (Fig. 342) club-shaped phragmometabasidium, (3–5)×(13–22) µm develops from the teliospore with lateral and terminal basidiospores, which are either *A1* or *A2* mating types.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	w	DL-Lactate	–
D-Xylose	+	Succinate	+/w
L-Arabinose	+	Citrate	+/w
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 10 (Yamada and Kondo 1973).

Mol% G+C: 67.3 (T_m: Nakase and Komagata 1971c), 64.8–66.1 (T_m: Hamamoto et al. 1986b).

Cell hydrolyzates: Xylose absent (Sugiyama et al. 1985).

Origin of the strains studied: Marine sources in southern Florida (14) including water over a southeast Florida reef with a water temperature of 29°C and a salinity of 36.2‰ and from mangrove detritus in the Florida Everglades, water temperature 20°C, salinity 19‰ (Newell and Hunter 1970).

Complementary mating types: CBS 6084 (Newell strain FR 4, mating type *A2*) and CBS 6085 (Newell strain FR 5, mating type *A1*). Both strains were collected from water over a southeast Florida reef.

Type strain: CBS 6085.

Comments: *R. diobovatum* is prevalent in near shore marine waters of South Florida, the Caribbean and the Bahamas. The species is often found in the vicinity of coral reefs (Fell and Statzell-Tallman, unpublished data). The species was reported from terrestrial plants and soils (Kvasnikov et al. 1975, Bab'eva and Kartintsev 1974), although those strains may belong to the newly described *R. babjevae* (Golubev 1993). Strains of *R. diobovatum* fit the description of *Rhodotorula glutinis,* although the type strain of *Rhodotorula glutinis* does not mate with either mating type of *R. diobovatum.* Nucleotide sequence analysis of a partial region of the LSU rRNA indicates that these two species are distinct and closely related (Fell et al. 1992). Barnett et al. (1990) list *Torulopsis terrestris* Verona (CBS 994) as a synonym of *R. diobovatum,* however, that strain was not included in the present study.

86.4. *Rhodosporidium fluviale* Fell, Kurtzman, Tallman & Buck (1988)

Growth in 5% malt extract: After 3 days at 25°C, cells are ovoidal to spheroidal, (3.5–8.0)×(5.4–9.4) µm. Cells occur singly or in pairs. After one month there is a heavy sediment and a light ring, but not a pellicle.

Growth on 5% malt agar: After 3 days at 25°C, cells are similar to those in malt extract with a few larger cells to 10.5 µm. The streak culture is light scarlet orange, smooth, glistening, slightly raised and the border is entire. Teliospores form in patches at the edge of the colony and they are slightly larger than those formed on corn meal agar.

Dalmau plate cultures on corn meal agar: After 6 days at 25°C, true mycelium forms with incomplete (rarely complete) clamps at the septa and at the teliospores. Nuclear stains indicate that the hyphae are uninucleate suggesting that formation of teliospores is the result of monokaryotic fruiting. The teliospores are spheroidal to subglobose, (7.4–10.7)×(8.7–12.1) µm, usually single or in pairs. A short, hyphal-like tip may form on the teliospores. In month old cultures, teliospores form in groups of up to 12. The walls are smooth but may become warty in older cultures. Teliospores are intercalary, rarely terminal or lateral on short branches.

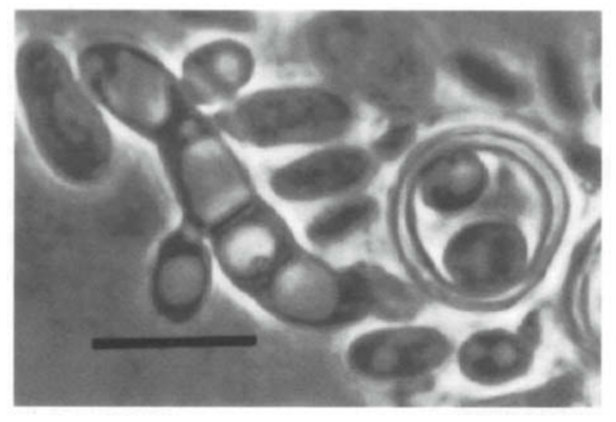


Fig. 343. *R. fluviale*, CBS 6568. Teliospore, which formed on corn meal agar at 25°C within one month, germinated to a 4-celled metabasidium with lateral basidiospores, after soaking for 2 weeks in deionized water. Bar = 10 μm.

Life history: Thick-walled teliospores form on the hyphae in the apparent absence of mating. These teliospores may germinate after one month on corn meal agar; germination in higher frequencies occurs after soaking 1-month-old teliospores for 2 weeks in distilled water followed by transfer to 2% aqueous agar. The resulting metabasidium is club-shaped (Fig. 343), consisting of 4 cells that break up into individual cells (a characteristic also observed in *R. sphaerocarpum*). Ovoid basidiospores, (~2.5×4.0) μm, form laterally on the metabasidium on short sporophores. Single basidiospores isolated by micromanipulation develop into homokaryotic fruiting strains similar to the parental strain.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	s	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	ws
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	w		

Co-Q: Not determined.
Mol% G + C: 61.4 (BD: Fell et al. 1988).
Cell hydrolyzates: Not determined.

Origin of the strains studied: Collected in October 1972, from brackish water of the Miami River, Florida (Fell et al. 1988).

Type strain: CBS 6568 (Fell strain ML 1580, NRRL Y-12922).

Comments: This species has only been isolated from one location. Nucleotide sequence analysis of a partial region of the LSU rRNA (Fell et al. 1992) suggests that *R. fluviale* may be related to strains of *Sporidiobolus ruineniae*.

86.5. *Rhodosporidium kratochvilovae* Hamamoto, Sugiyama & Komagata (1988a)

Growth on 5% malt extract: After 3 days at 25°C, the cells are encapsulated, ovoidal, (3–6)×(4–10) μm, occurring singly and in pairs. Growth is pink. After one month, a ring and sediment are present.

Growth on 5% malt extract agar: After one month at 25°C, the colony is peach- to orange-colored, slightly raised, the surface is semiglossy becoming rugose, while the margin is smooth, entire and fringed with true mycelium. The mycelium, which develops within one week, bears teliospores that are intercalary, terminal, or lateral on short stalks and false clamps at the septa.

Dalmau plate culture on corn meal agar: After one week at 19°C, septate hyphae have formed bearing false clamps and spheroidal or obpyriform teliospores (7–9 μm).

Life cycle: Mycelium with teliospores and false clamps at the septa develops from a single cell. Hamamoto et al. (1988a) reported that the hyphal cells are uninucleate and as growth occurs one nucleus remains in the incomplete clamp and one in the subterminal cell. They found that the teliospores were uninucleate and rarely germinated when 10-day-old cultures were soaked for 10 days in distilled water at 17°C followed by transfer to 2% aqueous agar for 10 days. Our investigations found that germination would occur after incubating for 8 weeks at 19°C on corn meal agar. A four-celled metabasidium (20–37)×(4–5) μm formed that produced lateral and terminal basidiospores, one basidiospore per metabasidial cell (Fig. 344).

Fermentation: absent.

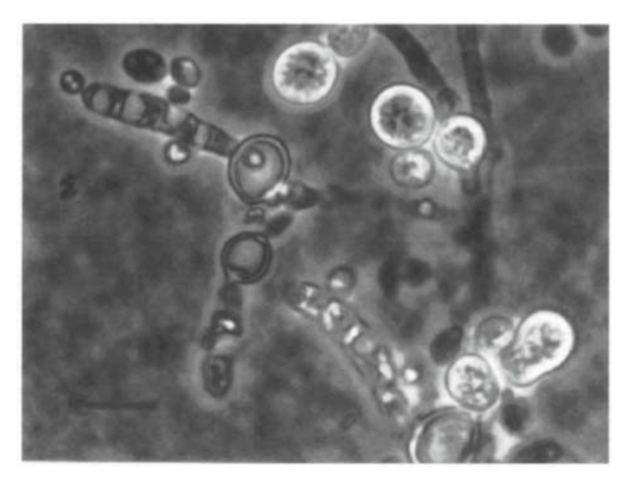


Fig. 344. *R. kratochvilovae*, CBS 7436. Teliospore formation after 7 days at 19°C on corn meal agar and germination after 2 months. Bar = 10 µm.

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	s	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w
D-Arabinose	+	Inositol	–
D-Ribose	w	Hexadecane	–
L-Rhamnose	s	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% Glucose–yeast extract agar	n	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G+C: 64.7–64.9 (T_m: Hamamoto et al. 1986b).
Cell hydrolyzates: Xylose absent (Sugiyama et al. 1985).

Origin of the strains studied: A record of the original isolation data of this species was not found. Two strains were included in the original description: the type strain and CBS 7293 (IAM 13073, CCY 62-3-2, YK 219).

Type strain: CBS 7436 (IAM 13072, CCY 62-3-1, YK 218).

Comments: Our results of assimilation tests based on CBS 7436 differed from the original description on cellobiose, D-xylose, L-arabinose and galactitol. Our results agreed with those of Barnett et al. (1990) who studied CBS 7293. Nucleotide sequence analysis of a region of LSU rRNA (Fell et al. 1992) indicates that *R. kratochvilovae* is related to *Rhodosporidium diobovatum* and *Rhodotorula glutinis* which is in agreement with the similarities in physiological characteristics of these species.

86.6. *Rhodosporidium lusitaniae* A. Fonseca & Sampaio (1992)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal or bacilliform, measure (2.5–5.9)×(5.9–20.1) µm, and occur in pairs or chains of three to four. There is a sediment present. After one month, neither a ring nor pellicle was observed.

Growth on 5% malt extract agar: After one month at 25°C, the colony is peach-colored, the surface is glossy and smooth, the texture is soft, the cross section is slightly raised and the border is fringed with mycelium. The latter bears clamps at the septa and spheroidal teliospores that may be intercalary, lateral on stalks or terminal. The teliospores are 9.5–13.3 µm in diameter and occur singly or in clusters of two to four.

Dalmau plate culture on corn meal agar: After one month at 25°C, true mycelium with teliospores and clamp connections form in abundance.

Life cycle (as described by Fonseca and Sampaio 1992): The life cycle was observed on corn meal agar and potato dextrose agar. Mating between cells did not take place. Uninucleate cells germinated to produce dikaryotic hyphae with complete clamp connections and terminal, intercalary and lateral uninucleate teliospores (Fig. 345).

Teliospores were transferred in small pieces of the agar to demineralized water and allowed to soak for 2 weeks at room temperature. At this time slender protrusions (measuring 5–20 µm) arose from the teliospores. The teliospores were transferred to 2% agar and, after 2 months at room temperature, 2–4-celled metabasidia (3.2–4.3×16–22 µm) emerged from the protrusions. Photographs by Fonseca and Sampaio (1992) show the teliospore with an elongate stalk terminated by a 4-celled

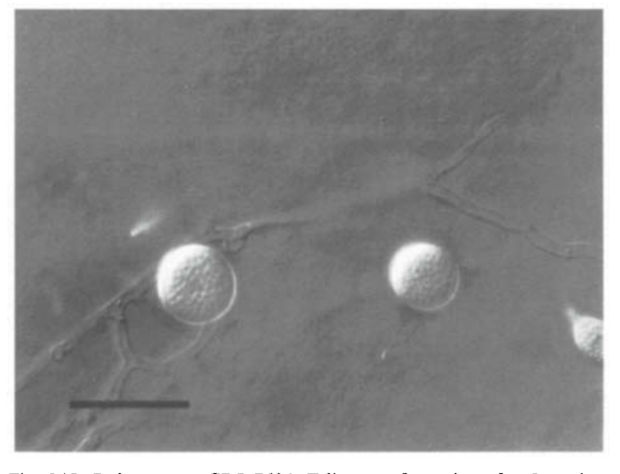


Fig. 345. *R. lusitaniae*, CBS 7604. Teliospore formation after 2 weeks on corn meal agar at 19°C. Bar = 10 µm.

metabasidium. One to four basidiospores (2.2–2.7×4.3–8.1 μm) arose from a single site at each cell of the phragmometabasidium.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	s	Succinate	+/w
L-Arabinose	s	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Veratryl alcohol	–
5-Keto-D-gluconate	n	Vanillic acid	+
Saccharate	–	Veratric acid	–
D-Glucuronate	–	Ferulic acid	+
Arbutin	+	*p*-Hydroxybenzoic acid	+
Cadaverine	–	*m*-Hydroxybenzoic acid	+
Creatine	–	Protocatechuic acid	+
Creatinine	–	*p*-Coumaric acid	+
L-Lysine	+/w	Caffeic acid	+
Ethylamine hydrochloride	+	Cinnamic acid	–
50% Glucose–yeast extract agar	–	Syringic acid	–
		Sinapic acid	–
10% NaCl/5% glucose	+	Gallic acid	+
Starch formation	–	Salicylic acid	–
Urease	+	Gentistic acid	–
Sodium nitrite	+	Guaiacol	–
Gelatin liquefaction	–	0.01% Cycloheximide	–
Glucono-δ-lactone	+	Growth at 30°C	+
L(+)-Tartaric acid	+	Growth at 37°C	–
Malic acid	+		
Vanillyl alcohol	–		

Co-Q: 9 (Fonseca and Sampaio 1992).
Mol% G+C: 62.0–62.1 (T_m: Fonseca and Sampaio 1992).
Cell hydrolyzates: Xylose absent (Fonseca and Sampaio 1992).

Origin of the strains studied: Fonseca and Sampaio (1992) isolated three strains: IGC 4641 from soil collected in a wood, Lisbon, Portugal, Spring 1987; IGC 4599 and IGC 4642 from dried leaf material collected in a natural park, south of Lisbon (Arrábida) in the summers of 1989 and 1990.

Type strain: CBS 7604 (IGC 4641).

Comments: In contrast to our results, Fonseca and Sampaio (1992) reported that the species was unable to utilize L-arabinose and cellobiose. The strains were isolated using media containing lignin-related phenolic compounds as the sole carbon source.

86.7. *Rhodosporidium malvinellum* Fell & I.L. Hunter (Fell 1970b)

Synonym:
Kondoa malvinella (Fell & I.L. Hunter) Y. Yamada, Nakagawa & Banno (1989a)

Growth in 5% malt extract: After 3 days at 12°C, the ovoidal to spheroidal haplophase cells measure (2–7)×(3–7) μm; they are single, occasionally in pairs with budding on short necks. A light sediment is formed. After one month there is a moderate sediment.

Growth on 5% malt agar: After 3 days at 12°C, the cell sizes are as above. The streak is creamy white, smooth and semi-glistening. The periphery is entire and the cross section is raised. At 2 weeks, the streak is light mauve and at one month the color has intensified.

Dalmau plate culture on corn meal agar: Pseudomycelium is generally absent; occasionally short branched chains of cells are present.

Life cycle: The species is heterothallic (biallelic, unifactorial); when compatible mating types are mixed on corn meal agar at 12°C, conjugation and binucleate mycelium with clamp connections are present within 3 days. Teliospores are rare after 7 weeks and abundant after 3 months. The teliospores are subglobose (5–10)×(7–12) μm. They develop singly and are attached to the hyphae by a short stalk (Fig. 346). Germination has only been observed once. The phragmometabasidium was 2-celled with lateral and terminal basidiospores.

Fermentation: absent.

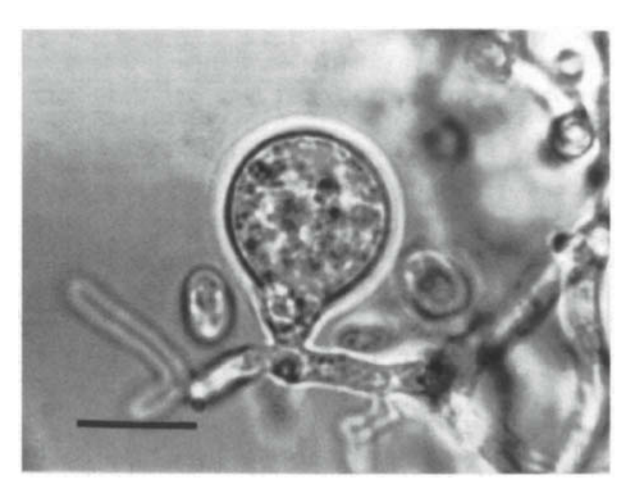


Fig. 346. *R. malvinellum,* CBS 6082×CBS 6083. Cells of opposite mating types were mixed on corn meal agar and grown at 12°C for 3 months; teliospores formed on a short stalk. Bar = 10 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	s	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	s
D-Xylose	s	Succinate	+
L-Arabinose	v	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	s	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Urease	+
5-Keto-D-gluconate	n	Gelatin liquefaction	–
Saccharate	–	PABA-free	–
D-Glucuronate	–	Thiamine-free	–
50% Glucose–yeast extract agar	–	Growth at 20°C	+
		Growth at 24°C	–
10% NaCl/5% glucose	–		
Starch formation	–		

Co-Q: 9 (Yamada and Kondo 1973).
Mol% G+C: 50.5 (T_m: Nakase and Komagata 1971c).
Cell hydrolyzates: Xylose absent (Sugiyama et al. 1985).

Origin of the strains studied: Marine waters of the southern Pacific, Indian and Antarctic Oceans (17) (Fell 1970b).

Complementary mating types: CBS 6082 (mating type *A1*), collected April 1966 at 60°29′S, 94°56′W at 53 m deep; CBS 6083 (mating type *A2*), collected on the same date and location at a water depth of 16 m with approximately the same temperature and salinity (Fell 1970b).

Type strain: CBS 6082.

Comments: The species appears to be restricted to marine habitats; in addition to the isolations from the southern oceans, Laurenavichene et al. (1989) isolated this species from coastal areas of the Baltic Sea. *R. malvinellum* has the physiological and life cycle characteristics typically found in other species of *Rhodosporidium*. The distinctive colony color is useful for species identification. Based on nucleotide sequence data the species does have distinctive genetic differences from other species of *Rhodosporidium*. Blanz and Gottschalk (1984, 1986) and Blanz and Unseld (1987) through examination of the nucleotide sequences of the 5S rRNA of 49 species of basidiomycetes found that *R. malvinellum* was more closely related to the Hymenomycetes, *Atractiella solani* and *Phleogena faginea*, than to *R. toruloides*. Blanz and Gottschalk suggested that *R. malvinellum* should be transferred to a separate genus. Yamada et al. (1989a) examined partial regions of the large and small subunits of rRNA of *R. malvinellum, R. toruloides, Leucosporidium scottii* and *Rhodotorula lactosa*. Based on the comparative differences in nucleotide sequences, Yamada et al. (1989a) described the genus *Kondoa* with the single species *Kondoa malvinellum*. Using the description of *R. malvinellum* published by Fell (1970b), Yamada et al. considered that *Kondoa* was morphologically unique through the production of teliospores attached by short stalks and 2-celled phragmometabasidia. However, other species, such as *R. toruloides* and *R. dacryoideum* produce lateral teliospores on short stalks or sporophores and their phragmometabasidia are 2–4-celled. Whether or not 2-celled phragmometabasidia are characteristic of *R. malvinellum* is unknown as germination has only been observed one time (Fell 1970b). Yamada et al. (1989a) also point out that *R. malvinellum,* as a psychrophile, is distinct from the other species of *Rhodosporidium*. It should be noted that *R. malvinellum* was collected from low temperature environments and that psychrophilic characteristics are also found in other species (e.g., *Leucosporidium scottii, L. antarcticum, Mrakia frigida*) collected from cold habitats.

Although it is difficult to distinguish phenotypic differences between *Kondoa* and *Rhodosporidium,* the genetic differences were confirmed by Fell et al. (1992) who examined a partial region of the LSU rRNA for 117 species of basidiomycetous yeasts. Based on the species examined, *R. malvinellum* appears to be more related to species of *Bensingtonia* than to *Rhodosporidium* spp. This suggested relationship, and that presented by Blanz and Unseld (1987), indicates the need for more in depth examination of nucleotide sequence analyses and their phylogenetic significance at the generic level. For taxonomic purposes, the only distinctive phenotypic character appears to be color, and separation of genera based on shades of color does not seem either warranted or practical. Therefore, we have tentatively maintained the taxonomic binomial *R. malvinellum* with the suggestion that further investigations may confirm *Kondoa* as a distinct genus.

86.8. *Rhodosporidium paludigenum* Fell & Statzell-Tallman (1980a)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to elongate, (2–4)×(3–11) μm. They occur singly and in pairs. There is a light sediment. After one month, there is a heavy ring with light to heavy sediment.

Growth on 5% malt agar: After 3 days at 25°C, the cell morphology is similar to that in malt extract. The one-month-old streak culture is salmon-orange, smooth, glistening, slightly raised or rugose and the border entire.

Dalmau plate culture on corn meal agar: After 6 days at 25°C, there are short chains of budding cells, and short lengths of true mycelium may be present.

Life cycle: The species is heterothallic. When opposite

mating types were mixed on corn meal agar (prepared with 20‰ seawater) and incubated at 22–25°C, true hyphae, bearing spheroidal (13–17 µm diameter) teliospores, developed within 6 days. The teliospores were in clusters of 2 to 4, intercalary and rarely terminal on the hyphae. Teliospore germination after 4 weeks on corn meal agar, resulted in 2- to 4-celled metabasidia (Fig. 347). Basidiospores were of the two parental types, indicating a unifactorial sexual system. *R. paludigenum* does not mate with the other species included in the genus.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	s	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	s
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	s	Succinate	w
L-Arabinose	s	Citrate	w
D-Arabinose	+	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	s	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G + C: 64.9–65.3 (Fell et al. 1988).
Cell hydrolyzates: Xylose absent (Sugiyama et al. 1985).

Origin of the strains studied: Intertidal black rush (*Juncus roemerianus*) marsh and mangrove (*Rhizophora mangle*) swamp waters bordering Card Sound near Miami, Florida, April 1973 (3) (Fell and Statzell-Tallman 1980a); seawater, Florida by Ahearn (1).

Complementary mating types: CBS 6567 (mating type *A1*, Fell strain ML 2226) and CBS 6566 (mating type *A2*, Fell strain ML 2225).

Type strain: CBS 6566.

Comments: Haploid strains of *R. paludigenum* fit the description of *Rhodotorula graminis*, however, the type strain of *Rhodotorula graminis* does not mate with either mating type of *R. paludigenum*. Additionally, nucleotide sequence analysis indicates that the two species are distinct, but closely related.

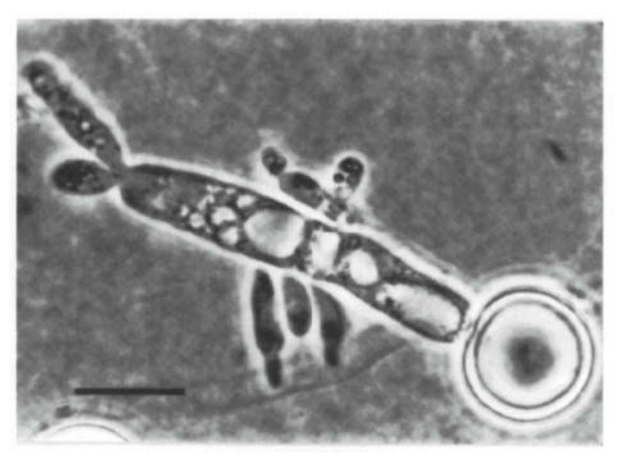


Fig. 347. *R. paludigenum*, CBS 6566×CBS 6567. Cells of opposite mating types were mixed and incubated on corn meal agar for 6 days; resulting teliospores germinated after 4 weeks to form a phragmometabasidium with lateral and terminal basidiospores. Bar = 10 µm.

86.9. *Rhodosporidium sphaerocarpum* Newell & Fell (1970)

Anamorph: *Rhodotorula glutinis* (Fresenius) Harrison var. *salinaria* Hirosawa & Takada

Synonym:
Rhodotorula glutinis (Fresenius) Harrison var. *salinaria* Hirosawa & Takada (1969)

Growth in 5% malt extract: After 3 days at 20°C, the cells are spheroidal to ellipsoidal, (3–5) × (5–7) µm, single or in pairs. Often newly divided cells form on very short necks <0.5 µm long. There is an orange to pink-orange sediment. After one month the sediment becomes thick and a weak ring has formed; spheroidal cells (7 µm) may be present.

Growth on 5% malt agar: Cell morphology at 20°C, is similar to that in malt extract. The streak culture at 3 days is smooth, glossy, orange to pink-orange; texture is highly mucoid; cross section is raised, spreading and the border is entire. After one month the culture becomes extremely mucoid; if kept at 5°C for one month, the culture does not become as mucoid as observed at 20°C, and develops a yellow pigmentation.

Dalmau plate culture on corn meal agar: Some strains produce no mycelium, others produce, from individual cells, short, fine, primary hyphae (diameter 1–3 µm) with 1 or 2 septa; self-sporulating strains develop extensive, larger (diameter 3.4–5.4 µm) septate hyphae, bearing large, spherical, thick-walled, terminal or intercalary teliospores.

Life cycle: *R. sphaerocarpum* has a biallelic unifactorial mating system. Mixing of haploid mating types *A1* and *A2* on corn meal agar at 25°C results in conjugation followed by hyphal development from the conjugation tube, or from one of the two conjugate cells (Fig. 348). The mycelium is dikaryotic; clamp connections form at the septa and thick-walled teliospores develop terminally and intercalary on the hyphae. The teliospores are at first binucleate and eventually become uninucleate. They are spheroidal,

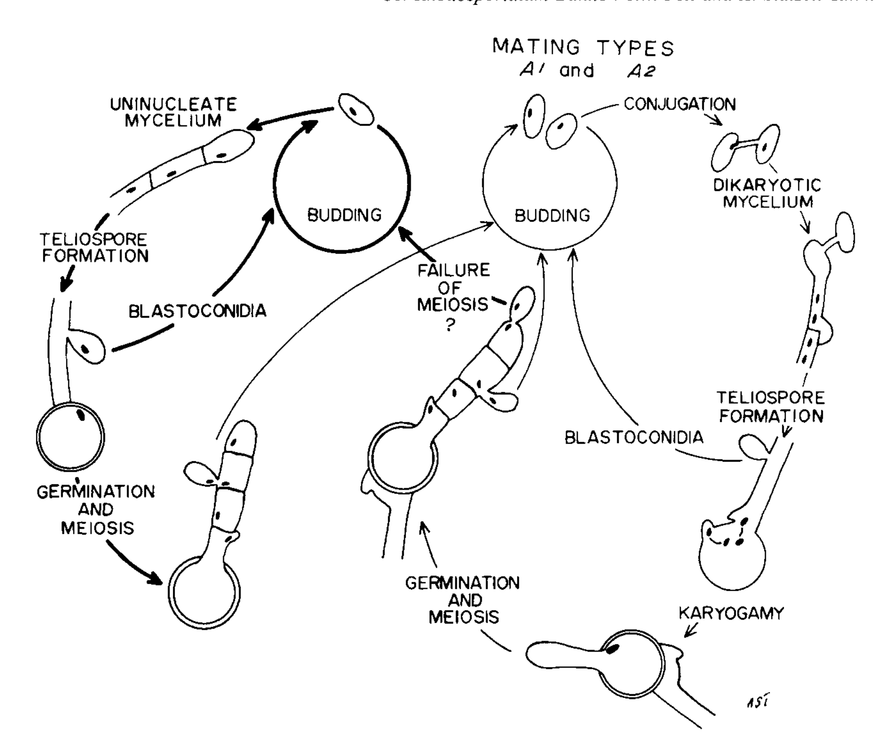


Fig. 348. Diagrammatic representation of the life cycle of *R. sphaerocarpum* (after Newell and Fell 1970).

smooth, and dark in color at maturity, occurring singly, in pairs, and in branched chains of as many as 12 or more. When teliospores are present in large quantities the culture becomes dark brown. The teliospores germinate if placed in distilled water at 25°C for one month followed by transfer to malt agar at the same temperature. The teliospore germinates to a 4-celled metabasidium, each cell capable of repeated mitotic divisions for the formation of several basidiospores. The metabasidium eventually breaks up into individual cells (Fig. 349). The basidiospores are haploid; a haplo-lethal effect occurs in some strains: examination of progeny from germination of teliospores indicated that all colonies were of mating type *A1*; apparently mating type *A2* did not survive.

Some strains produce teliospores in the absence of mating. The yeast cells are uninucleate and produce uninucleate teliospore-bearing hyphae without clamp connections. The mycelial formation is less extensive than the dikaryotic mycelium produced by the conjugating strains. Teliospores of the self-sporulating strains germinate under the same conditions as described above for the heterothallic strains to produce phragmometabasidia with haploid basidiospores (Fig. 347). Although Newell and Fell (1970) postulated that the haploid cells were the result of a meiotic division in the basidium, it is likely that these strains are homokaryotic fruiters similar to those of *Leucosporidium scottii*. Studies have not been undertaken to confirm either possibility.

Strains of *R. sphaerocarpum* fit the description of *Rhodotorula glutinis*: The type strain of *Rhodotorula glutinis* does not mate with mating types of *R. sphaerocarpum*. Similarly, nucleotide sequence analysis indicates that the two species are separate (Fell et al. 1995).

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	v
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	+
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Thiamine-free	–
D-Glucuronate	–	Growth at 27°C	+
50% Glucose–yeast extract agar	–	Growth at 30°C	w
		Growth at 33°C	–
10% NaCl/5% glucose	+		
Starch formation	–		

Co-Q: 10 (Yamada and Kondo 1973).

Mol% G+C: 64.9–65.4 (T_m: Nakase and Komagata 1971c); 64.0–64.1 (BD: Fell et al. 1988); 62.2–62.7 (T_m: Hamamoto et al. 1987).

Cell hydrolyzates: Xylose absent (Sugiyama et al. 1985).

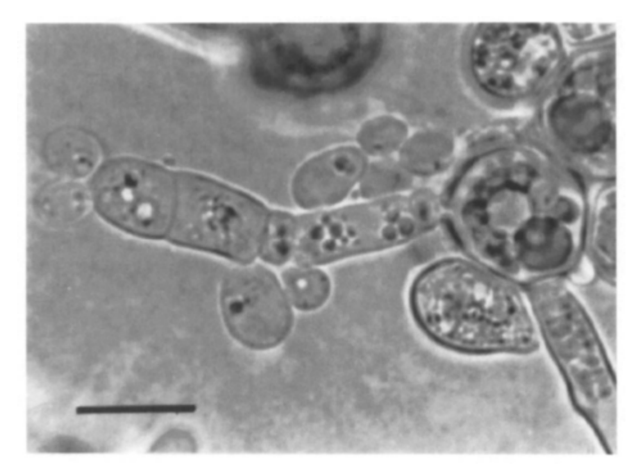


Fig. 349. *R. sphaerocarpum*, CBS 5939×CBS 5940. Teliospores formed from a mix of cells of opposite mating types after 2 weeks on corn meal agar at 25°C. After soaking in distilled water for 1 month, germination occurred within one day of placing the teliospores on V8 agar. Bar = 10 µm.

Origin of the strains studied: Marine sources near the Antarctic (Palmer) Peninsula and Palmer Archipelago, Jan.–Feb. 1966, from waters whose temperatures ranged from −1.68 to 1.33°C and salinities of 32.48‰ to 34.72‰ (33) and from the Caribbean Sea, Jan.–Feb. 1967, 100–150 miles off the coasts of Columbia and Panama, over the Columbian Basin in water depths of 184–297 meters where temperatures ranged from 12.90 to 17.72°C and salinities of 35.62 to 36.42‰ (4) (Fell et al. 1973); salt farm in 1967 at Yashima along the shore of Seto Inland Sea, Japan (1) (Hirosawa and Takada 1969).

Complementary mating types: CBS 5940 (Newell and Fell strain AR-74, mating type *A1*) collected from the Gerlache Straits near Anvers Island, Antarctica. CBS 5939 (Newell and Fell strain AR-3, mating type *A2*) collected from Marguerite Bay, Antarctica. Self-sporulating strain: CBS 5941 (Newell and Fell strain AR-77, strain from the Gerlache Straits near Brabant Island, Antarctica.

Type strain: CBS 5939.

Comments: *R. sphaerocarpum* is a commonly occurring species in marine habitats. In addition to the sources previously listed, we found this species to be prevalent in near shore waters of the Caribbean, Bahamas and Florida, often in association with coral reefs. J. Buck (personal communication) reports that *R. sphaerocarpum* is abundant in freshwater lakes in Connecticut, U.S.A.

86.10. *Rhodosporidium toruloides* Banno (1967)

Anamorph: *Rhodotorula rubescens* (Saito) F.C. Harrison

Synonyms:

Torula rubescens Saito (1922)
Mycotorula rubescens (Saito) Ciferri & Redaelli (1925)
Rhodotorula rubescens (Saito) F.C. Harrison (1928)
Rhodotorula glutinis (Fresenius) F.C. Harrison var. *rubescens* (Saito) Lodder (1934)
Rhodotorula longissima Lodder (1934)
Rhodotorula gracilis Rennerfelt (1937)
Rhodotorula glutinis (Freasenius) F.C. Harrison var. *rufusa* Iizuka & S. Goto (1965)

Growth in 5% malt extract: After 4 days at 25°C, heterothallic haploid cells are spheroidal to elongate, (2–7)×(4–12) µm. The homothallic cells are ovoidal to obtuse oblong, (3–7)×(6–30) µm, and may form true mycelium and teliospores.

Growth on 5% malt agar: After 4 days at 25°C, the cells are similar in size to those in malt extract. The streak culture is pink to orange, the border is entire, the surface is smooth and glistening, the texture is soft to slightly mucoid. After one month, the color becomes scarlet. The homothallic cultures have dark brown areas consisting of true mycelium and teliospores.

Dalmau plate culture on corn meal agar: After 2 weeks at 25°C, pseudomycelium of heterothallic strains may be absent or range from short branched chains of pseudomycelium to abundant clusters of pseudomycelium. The homothallic strains have extensive true mycelium with clamps and teliospores.

Life cycle: The species is homothallic and heterothallic (Fig. 350). Heterothallism: On malt agar at 25°C cells of compatible mating types (*A1* and *A2*) conjugate within 24 hours and plasmogamy occurs. From one cell of the conjugate pair, a septate hypha develops with clamp connections at the septa. Angular, irregularly shaped, thick-walled teliospores develop terminally or laterally on short, clamped sporophores (Fig. 351).

After 1–2 weeks the spores germinate to produce club-shaped metabasidia with 2–4 cells. Basidiospores bud laterally and terminally. Some of the basidiospores are heterothallic and capable of mating and repeating the life cycle.

Homothallism: In addition to the heterothallic basidiospores, homothallic basidiospores are produced on the metabasidium. These are uninucleate cells that develop directly into a dikaryotic mycelium with clamp connections and teliospores. The teliospores germinate to produce

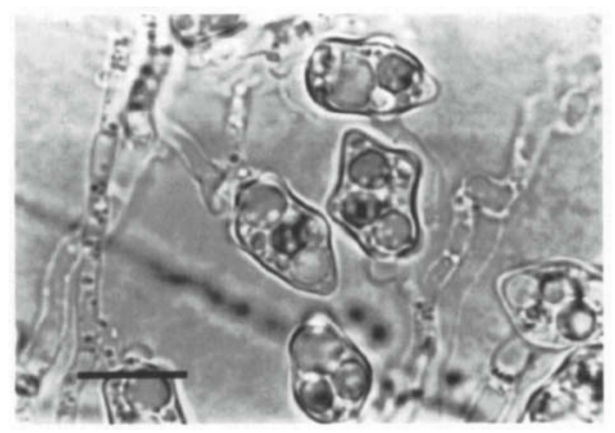


Fig. 351. *R. toruloides*, CBS 14×CBS 349. Teliospores formed from a mix of cells of opposite mating types on corn meal agar at 25°C after 2 weeks. Bar = 10 µm.

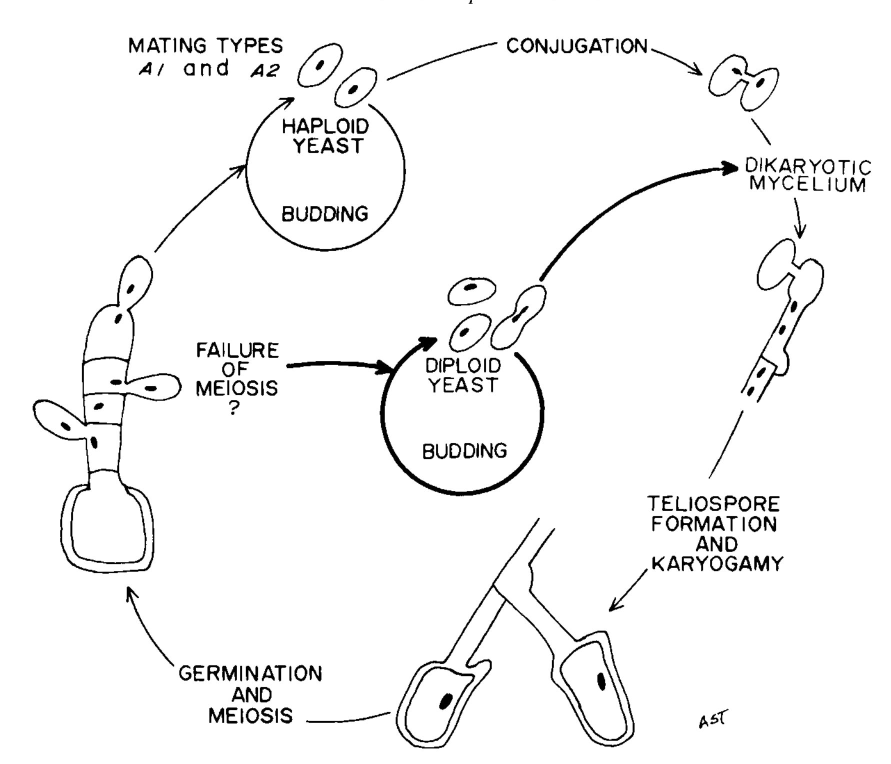


Fig. 350. Diagrammatic representation of the life cycle of *R. toruloides* (Fell et al. 1970).

metabasidia with both heterothallic and homothallic basidiospores.

The heterothallic and homothallic basidiospores are uninucleate, the hyphae with clamp connections, produced by both heterothallic and homothallic phases, are binucleate. The teliospores are initially binucleate, and become uninucleate through karyogamy. At germination the diploid nucleus undergoes reduction division into the four haploid nuclei of the metabasidium. Anamorphic strains of *R. toruloides* fit the description of *Rhodotorula glutinis*. The type culture of *Rhodotorula glutinis* does not mate with mating types of *R. toruloides*.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	s	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	w
L-Arabinose	+	Citrate	w
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Urease	+
Saccharate	–	Gelatin liquefaction	v
D-Glucuronate	–	Growth at 30°C	+
50% Glucose–yeast extract agar	–	Growth at 37°C	w
10% NaCl/5% glucose	+		

Co-Q: 9 (Yamada and Kondo 1973).

Mol% G+C: 60.0–61.0 (T_m: Nakase and Komagata 1971c, Nakase 1972b); 59.3–60.5 (T_m: Hamamoto et al. 1987).

Cell hydrolyzates: Xylose absent (Sugiyama et al. 1985).

Origin of the strains studied: Conifer pulp in Sweden (CBS 14) (Rennerfelt 1937); soil in Japan (CBS 349) (Okunuki 1931); air, Japan (CBS 315, type strain of *Torula rubescens* Saito; Southern Pacific Ocean (Fell strain 27-51, 1967); gut of a dead porpoise, Bimini, Bahamas (2); unknown (2).

Complementary mating types: IFO 0559 (CBS 14) mating type *A1* and IFO 0880 (CBS 349) mating type *A2*.

Type strain: Banno designated the mycelial conjugate of IFO 0559 (CBS 14) and IFO 0880 (CBS 349) as the type strain. He deposited the conjugate in the IFO collection as IFO 11588. We recommend the living type strains as the holotype CBS 14 (mating type *A1*) and the allotype CBS 349 (mating type *A2*). Self-sporulating strain: CBS 6016 (IFO 8766).

Comments: In addition to the sources listed above, we isolated the species from tropical marine waters in South Florida, the Caribbean and Bahamas, usually in regions of coral reefs. The species, although widespread in the marine habitats, was not abundant. *Rhodotorula*

glutinis var. *rufusa,* an anamorph of *R. toruloides,* was isolated (Iizuka and Goto 1965) from soil of the Nishiyama oil field, Nirgata Prefectura, Japan. Joo et al. (1988) found a colorless variant of *R. toruloides* and questioned the use of color in separating *Leucosporidium* from *Rhodosporidium.* Sequence analysis of the LSU rRNA indicates that these two genera are phylogenetically distinct (Fell et al. 1992).

Comments on the genus

A sexual life cycle involving mating between two strains of *Rhodotorula glutinis* was first reported by Banno (1967) with the description of the genus *Rhodosporidium* and the species *R. toruloides.* Although a basidiomycetous life cycle had been established for *Sporidiobolus johnsonii* Nyland, Banno's observation confirmed the basidiomycetous nature of strains of *Rhodotorula* and opened the door to further exploration into sexual cycles in *Rhodotorula.* Nine additional species followed and we predict that many more will be found. This prediction is based on the presence of numerous anamorphic strains and species of *Rhodotorula* with unknown teleomorphic states.

Some *Rhodosporidium* species (*R. babjevae, R. kratochvilovae, R. diobovatum, R. paludigenum* and *R. toruloides*), based on sequence analysis of the large subunit rDNA, are related to the anamorphs *Rhodotorula graminis, R. araucariae, R. mucilaginosa* and *R. glutinis.* Mating reactions between these teleomorphs and anamorphs has not been observed. Another species, *R. fluviale,* appears related to *Sporidiobolus ruineniae.* Sequence analysis indicates that *R. dacryoideum* and *R. malvinellum* are not related to the above mentioned species, instead *R. malvinellum* appears close to *Bensingtonia yuccicola,* and *R. dacryoideum* is in a large cluster of species that includes *Rhodotorula lactosa* and *Erythrobasidium hasegawae* (Fell et al. 1992, 1995). Because of these nucleotide sequence differences, recommendations have been made (Yamada et al. 1989a, 1994a) to create new genera for *R. malvinellum* (*Kondoa*) and *R. dacryoideum* (*Sakaguchia*). The difficulty with these recommendations is that the genera do not have sufficient classical taxonomic differences to separate them from *Rhodosporidium,* or distinguish them as separate genera. The paramount character is derived from nucleotide sequence analysis, which currently is an impractical technique for routine identification. Therefore, we have maintained the present integrity of *Rhodosporidium,* recognizing the polyphyletic nature of the genus. As our knowledge of the relationships of species and genera in the Heterobasidiomycetes becomes more acute, a stronger basis for generic separations will emerge. Currently, species within the genus can readily be separated by teliospore shape, colony color and a limited number of assimilation tests (Table 70).

87. *Sporidiobolus* Nyland

A. Statzell-Tallman and J.W. Fell

Diagnosis of the genus

Colony color on solid media includes shades of pink and red due to the presence of carotenoid pigments. Vegetative reproduction by budding, formation of pseudomycelium and true mycelium, and by ballistoconidia. Ballistoconidia develop on simple and branched sterigmata that arise from yeast and mycelial cells. The septal structure consists of a simple pore.

Sexual mechanisms are homothallic and heterothallic. In the heterothallic system a dikaryotic mycelium results from the conjugation of a compatible mating pair. A clamp connection is usually present at each cross wall. Intercalary and terminal teliospores develop. Karyogamy takes place in the teliospore (probasidium) which germinates to produce a metabasidium (promycelium). Meiosis takes place during germination with the formation of haploid basidiospores.

Homothallism consists of the development of hyphae with teliospores from a single yeast cell in the absence of mating. The yeast phase is diploid; reduction division takes place with the formation of a dikaryotic hypha with clamp connections. Karyogamy occurs in the teliospore. Germination of the teliospore results in a metabasidium with diploid basidiospores. Strains of some species produce teliospores on uninucleate hyphae that lack clamp connections (self-sporulation). The ploidy of the nucleus is unknown.

The metabasidia are one-celled (holometabasidia) or septate with two or more cells (phragmometabasidia). The basidiospores are terminal on the holometabasidium and lateral and terminal on the phragmometabasidium. In some species the metabasidium is connected to the teliospore by an aseptate stalk that ranges in length from 3 to 26 μm.

Sugars are not fermented, starch formation is negative, Diazonium blue B and urease are positive, xylose is absent from cell hydrolyzates, D-glucuronate and *myo*-inositol are not assimilated. Coenzyme Q-10 is present.

Type species

Sporidiobolus johnsonii Nyland

Species accepted

1. *Sporidiobolus johnsonii* Nyland (1949)
2. *Sporidiobolus pararoseus* Fell & Tallman (1981)
3. *Sporidiobolus ruineniae* Holzschu, Tredick & Phaff (1981)
4. *Sporidiobolus salmonicolor* Fell & Tallman (1981)

Key to species

See Table 71.

1.	a Homothallic formation of teliospores present → 2			
	b Homothallic formation of teliospores absent → 4			
2(1).	a Nitrate assimilated → 3			
	b Nitrate not assimilated	....	*S. pararoseus*:	p. 694
3(2).	a Melezitose assimilated	....	*S. johnsonii*:	p. 694
	b Melezitose not assimilated	....	*S. ruineniae*:	p. 696
4(1).	a Nitrate assimilated	....	*S. salmonicolor*:	p. 697
	b Nitrate not assimilated	....	*S. pararoseus*:	p. 694

Table 71
Key characters of the species in the genus *Sporidiobolus*

Species	Teliospore formation			Assimilation	
	Homothallic	Self-sporulating	Heterothallic	Melezitose	Nitrate
Sporidiobolus johnsonii	+	–	–	+	+
S. pararoseus	–	+	+	+	–
S. ruineniae	+	+	–	–	+
S. salmonicolor	–	–	+	v	+

Systematic discussion of the species

87.1. *Sporidiobolus johnsonii* Nyland (1949)

Anamorph: *Sporobolomyces holsaticus* Windisch ex Yarrow & Fell
Synonyms:
Prosporobolomyces holsaticus (Windisch) Novák & Zsolt (1961)
Sporobolomyces holsaticus Windisch ex Yarrow & Fell (1980)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal and elongate, (2–7)×(5–27) µm, single, in pairs and in short chains. The cells are budding and may have simple or branched sterigmata bearing kidney-shaped ballistoconidia, (4–7)×(8–14) µm. Short hyphal strands develop from single cells. A thick, dull, corrugated grenadine-pink pellicle or a thick ring and a light sediment are present. After one month, there is a moderate sediment and a strongly wrinkled pellicle. There may be extensive formation of mycelium with complete clamp connections and teliospores.

Growth on 5% malt agar: After 5 days at 25°C, the streak culture is yellowish-pink colored with a convoluted surface. The streak is shiny in the center to dry and powdery at the periphery; true mycelium forms at the edge of the colony. After one month the colony is peach-colored. By 3 weeks, there is an extensive formation of mycelium with clamps and teliospores.

Dalmau plate culture on corn meal agar: After 5 days at 25°C, there are budding yeast cells as well as cells with simple and branched sterigmata bearing ballistoconidia; extensive true mycelium with clamp connections and spherical teliospores, 10–15 µm in diameter may be produced.

Formation of ballistoconidia: Asymmetric, reniform, (2–4)×(5–10) µm, ballistoconidia form on simple or branched sterigmata after 3 days on malt agar or corn meal agar.

Life cycle: As described by Laffin and Cutter (1959a,b) the species is homothallic. The yeast phase is diploid; reduction division occurs with the formation of dikaryotic hyphae with clamp connections. Karyogamy occurs in the teliospore. Germination of the teliospore results in a holometabasidium with terminal diploid basidiospores. To obtain germination, the cultures were grown on malt agar (22°C) for 4 weeks and then the teliospores were transferred to 2% agar where they germinated after 10 days of incubation.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	v
D-Xylose	ws	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	+	Inositol	–
D-Ribose	s	Hexadecane	s
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	10% NaCl/5% glucose	+
5-Keto-D-gluconate	–	Starch formation	–
Saccharate	–	Urease	+
D-Glucuronate	–	Gelatin liquefaction	w/–
Arbutin	+	Growth at 30°C	+
50% Glucose–yeast extract agar	–	Growth at 37°C	v

Co-Q: 10 (Yamada et al. 1983, Nakase and Suzuki 1986d).
Mol% G+C: 63.5 (BD: Holzschu et al. 1981); 62.0, 64.0 (BD: Storck et al. 1969).
Cell hydrolyzates: Mannose, glucose, galactose, fucose (von Arx and Weijman 1979).

Origin of the strains studied: Pustule of *Phragmidium rubi-idaei* (DC.) Karst. on a living leaf of *Rubus idaeus* L. in the State of Washington, U.S.A. (1) (Nyland 1949); fodder yeast (1) from Windisch 1949.

Type strain: CBS 5470 (Nyland 1949).

Comments: The majority of the strains labeled *Sporobolomyces holsaticus* that we studied were found to mate with strains of *Sporobolomyces salmonicolor*. One exception was the type strain of *S. holsaticus* (CBS 1522), which was maintained as a separate species in the last edition of "The Yeasts". Subsequent studies of CBS 1522 concluded that *S. holsaticus* is a synonym of *Sporidiobolus johnsonii* based on morphological and physiological similarities, DNA–DNA hybridization (93.1%) between the type strains of these species (Boekhout 1991a), the electrophoretic pattern of enzymes (Yamazaki and Komagata 1983a) and nucleotide sequence analysis (Fell et al. 1992). CBS 1522, therefore, represents an anamorphic strain of *S. johnsonii*, viz., hyphae with clamps and teliospores are not formed.

87.2. *Sporidiobolus pararoseus* Fell & Tallman (Fell and Statzell-Tallman (1981)

Anamorph: *Sporobolomyces shibatanus* (Okunuki) Verona & Ciferri
Synonyms:
Torula shibatana Okunuki (1931)
Sporobolomyces pararoseus Olson & Hammer (1937) nom. nud.
Sporobolomyces shibatanus (Okunuki) Verona & Ciferri (1938)

Sporobolomyces marcillae Santa María (1958b)
Prosporobolomyces marcillae (Santa María) Novák & Zsolt (1961)
Sporobolomyces ruber Yamasaki & Fujii (1950)
Sporobolomyces carnicolor Yamasaki & Fujii (1950)
Sporobolomyces japonica Iizuka & Goto (1965)

Growth in 5% malt extract: After 3 days at 25°C, there is considerable variation in cell shape; the cells may be ovoidal, spherical, elongate or allantoid, (3–9)×(4–21) μm. They occur singly, in pairs, small clusters and short chains. A thin ring and light to moderate sediment are present. After one month a thick corrugated pellicle may form and the sediment may be heavy.

Growth on 5% malt agar: After one month at 25°C, the streak culture is pale salmon, grenadine, coral-red or peach-red in color with a rugose or smooth, glossy or semidull surface. The texture is soft. The cross section is low convex to flat and the border entire, occasionally with a mycelial fringe.

Dalmau plate culture on corn meal agar: The strains vary considerably; pseudo- and true hyphae are absent in some strains, whereas other strains will produce light to extensive amounts of both.

Formation of ballistoconidia: Although some strains form ballistoconidia on corn meal agar, Phaff (1970c) found that a medium, which consisted of Yeast Nitrogen Base agar with either ribose, glycerol or glucono-δ-lactone was more suitable for ballistoconidium production for other strains. The ballistoconidia are asymmetric, measuring (2–5)×(5–12) μm, with 1–5 or more sterigma.

Life cycle: When compatible mating types were mixed on corn meal agar at 22°C, conjugation was followed by formation of hyphae with hook-to-peg clamps and teliospores. The latter are elliptic-fusiform or spherical, (9–13)×(13–24) μm, occurring singly, in pairs or rarely in chains of three. After 35 days on corn meal agar at 22°C, the teliospores germinated on the corn meal agar by forming branched hyphae with buds. Continued growth of either the buds or hyphae was not successful. Two mating types were found, suggesting a unifactorial mating system with two alleles.

One strain, identified as *S. pararoseus* (CBS 5541), produced extensive true mycelium with elliptic-fusiform to spherical teliospores, (10–12)×(12–20) μm. The teliospores were single and in chains, located intercalarily and terminally on the hyphae and on short lateral branches. Clamp connections were not formed. This self-sporulating strain did not mate with either the *A1* or *A2* mating types of *S. pararoseus*. DNA sequence analysis indicates that this strain maybe related to *Sporobolomyces roseus* rather than to *S. pararoseus*.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	s
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	v
D-Xylose	v	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	w	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Yamada and Kondo 1973).
Mol% G+C: 50.0–53.7 (T_m: Boekhout 1991b); 51.5–55.0 (BD: Storck et al. 1969).
Cell hydrolyzates: Glucose, mannose, galactose and fucose (Gorin and Spencer 1970).

Origin of the strains studied: Sources include soil (Okunuki 1931), air (Olson and Hammer 1937), flower, plants and seawater.

Complementary mating types: CBS 491, mating type *A2*, and CBS 499 (Olson and Hammer 1937), mating type *A1*, from air in a dairy, USA, isolated by B.W. Hammer. Additional mating strains: CBS 484, mating type *A1*, type strain of *Sporobolomyces pararoseus,* from air, USA, B.W. Hammer; CBS 4216, type strain of *Sporobolomyces ruber* Yamasaki & Fujii, mating type *A2*; CBS 5329 and CBS 5330, mating type *A2,* from seawater in Atlantic Ocean, USA by D. Ahearn. Self-sporulating strain: CBS 5541, from flower of *Fumaria* sp., France by J. Villoutreix.

Type strain: CBS 491, type strain of *Sporobolomyces shibatanus,* mating type *A2*, from soil, Japan, isolated by K. Okunuki.

Comments: Two strains identified as *Sporobolomyces pararoseus,* CBS 4217 (type strain of *S. marcillae* and CBS 2637, did not mate with either of the mating types of *S. pararoseus.* Storck et al. (1969) found that the G+C content of the type strain of *S. pararoseus* (CBS 484) is 51.5%, whereas the G+C content of CBS 4217 is 55.0% and CBS 2637 is 60.5%. Apparently these two strains belong to one or more species distinct from *S. pararoseus.*

87.3. *Sporidiobolus ruineniae* Holzschu, Tredick & Phaff (1981)

Synonyms:

Sporobolomyces coprophilus Sugiyama & S. Goto (1967)

Sporidiobolus ruineniae Holzschu, Tredick & Phaff var. *coprophilus* Kurtzman & Fell (1991)

Sporidiobolus microsporus Higham (1970) nom. nud.

Growth in 5% malt extract: After 3 days at 25°C, the cells are cylindrical or ovoidal, (2–9)×(6–13) μm, and single or in pairs. Some cells have sterigmata with several bud scars. Short hyphal strands develop from single cells. A light ring and a moderate pellicle may be present. Hyphae with clamp connections and spherical teliospores (4–20 μm in diameter) develop.

Growth on 5% malt agar: After 3 days at 25°C, the streak culture is cream to light buff or peach-colored (see comments). The surface is smooth, rugoid, slightly raised, and slightly glistening. The border is entire with tufts of mycelium formed by some strains; clamp connections (incomplete, rarely complete) may or may not be present on the mycelium; teliospores may occur. After one month, there is a more extensive development of hyphae and teliospores and the colony is orange-luteolus or peach-red.

Dalmau plate culture on corn meal agar: After 6 days at 25°C, true and pseudomycelium may be present. By 6 weeks, teliospores develop on true mycelium. Complete or incomplete clamp connections may be present; spherical teliospores are present that resemble those on malt agar.

Formation of ballistoconidia: Asymmetric ballistoconidia, (2–7)×(6–10) μm, form on most growth media, with the exception of one strain (CBS 5811, *Sporobolomyces coprophilus*) that appears to have lost this ability.

Life cycle: The species is homothallic; teliospores develop from single cells in the absence of mating. *S. ruineniae* and *S. microsporus* strains develop hyphae with complete and incomplete clamp connections and germination of teliospores at 19°C results in a metabasidium that connects to the teliospore by an aseptate stalk, which ranges in length from 3–26 μm. Rates of teliospore germination vary. Teliospores from a streak culture of a *S. ruineniae* strain (Figs. 352, 353) on corn meal agar for 1.5 to 5 weeks, which were moved in a block of agar to distilled water for 4–12 weeks, germinated within 3 days of being transferred onto 2% aqueous agar; the *S. microsporus* strain teliospores, from a streak on a corn meal agar Dalmau plate for 8 weeks, were soaked for 2 weeks in distilled water; germination occurred after one week on 2% agar. The metabasidia were two- and four-celled with lateral and terminal basidiospores. Ploidy of the basidiospores was not determined. The third strain, *S. coprophilus,* produced teliospores on mycelium that lacked complete clamps, although sparse incomplete clamps were present. Teliospore germination occurred in a 7-week-old culture on malt agar, which was soaked for 2–4 weeks in distilled water and transferred to 2% agar for 3 days. The club-shaped phragmometabasidium lacked the aseptate basal cell present in the other two species (Fig. 354); the resulting basidiospores formed in two to four-spore clusters on a short pedicel and developed a parental-type life cycle: teliospores formed on hyphae that lacked clamp connections.

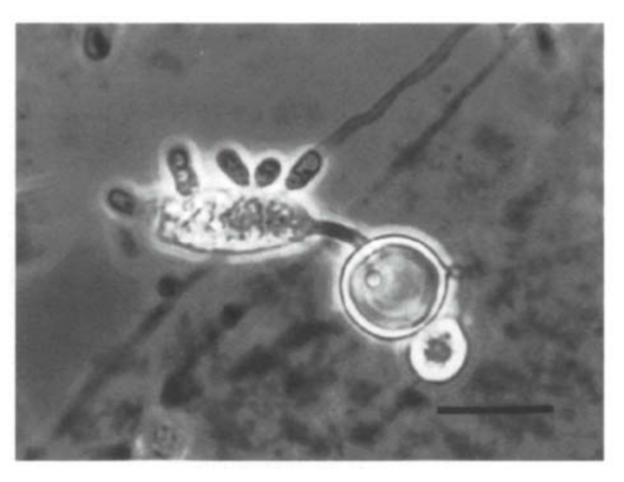


Fig. 352. *S. ruineniae,* CBS 5001. Teliospore, which formed from cells grown on corn meal agar at 19°C after 5 weeks, germinated after soaking in deionized water for 5 weeks. The 3-celled metabasidium is attached to the teliospore by an aseptate basal cell. Basidiospores, often in clusters of 2–4 cells, are on small pedicels. Bar = 10 μm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	s
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	+	Citrate	w
D-Arabinose	s	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% Glucose–yeast extract agar	w/–	Growth at 37°C	–
10% NaCl/5% glucose	s		

Co-Q: 10 (Nakase and Suzuki 1986d).

Mol% G+C: CBS 5811, 64.5; CBS 5001, 64.9 (BD: Holzschu et al. 1981; BD: Kurtzman and Fell 1991).

Cell hydrolyzates: Xylose absent (Suzuki and Nakase 1988a).

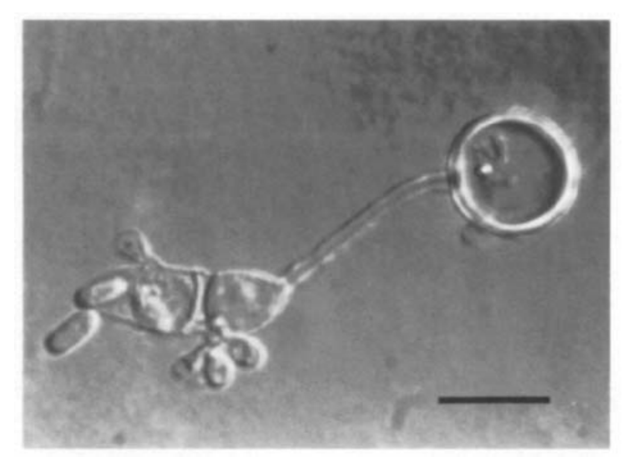


Fig. 353. *S. ruineniae*, CBS 5001. Teliospore, which formed on corn meal agar at 19°C after 5 weeks, germinated after soaking in deionized water for 5 weeks. The 2-celled metabasidium, is attached to the teliospore by an aseptate basal cell. Basidiospores, often in clusters of 2–4 cells, are on small pedicels. Bar = 10 µm.

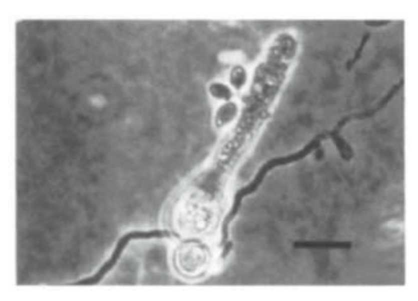


Fig. 354. *S. ruineniae* (*S. coprophilus* type strain), CBS 5811. Teliospore formed on malt agar after 7 weeks and then soaked in deionized water for 2 weeks. Germination to a club-shaped metabasidium lacking an aseptate basal cell occurred within 4 days on 2% aqueous agar. Bar = 10 µm.

Origin of the strains studied: Four strains of *S. ruineniae* (CBS 4998, 4999, 5000 and 5001, type) were isolated from the phyllosphere of tropical foliage of *Malphiga coccigera* at Bogor, Indonesia (Ruinen 1963). The type strain of *Sporobolomyces coprophilus* (CBS 5811) was isolated from goat dung in Pakistan (Sugiyama and Goto 1967). This strain lost the ability to produce ballistoconidia and was considered to be a strain of *Rhodotorula graminis* (Phaff and Ahearn 1970). *Sporidiobolus microsporus* (CBS 7041) was studied by June Higham (Higham 1970), a student of R.J. Bandoni, however, a formal description was not published (Bandoni, personal communication).

Type strain: CBS 5001 (isolated by Ruinen 1963).

Comments: The three species (*S. ruineniae, S. coprophilus* and *S. microsporus*) have been placed in synonymy (Boekhout 1991a) based on their similar physiological and morphological properties. Our morphological and molecular studies suggest that this decision should be examined in greater detail. *S. ruineniae* (Figs. 352, 353) and *S. microsporus* (no photograph included) strains have complete and incomplete clamp connections on the hyphae and their metabasidia form on stalks. In contrast the *S. coprophilus* strain has incomplete clamp connections (very rarely complete) and the few teliospores that germinated revealed a metabasidium that lacks a connecting stalk (Fig. 354). *S. microsporus* differs from the other strains by colony color, i.e., after 3 days on malt extract *S. microsporus* is cream to buff and at one month is orange-luteolus; the other strains are peach-colored with an increase in intensity to peach-red. *S. microsporus* does not utilize maltose; the other strains utilize maltose, sometimes slowly. Nucleotide sequence studies of a partial region of the LSU rRNA (Fell et al. 1992) indicate that the *S. ruineniae* and *S. coprophilus* strains have identical sequence alignments and that *S. microsporus* differs by 11 base positions (~4% of positions sequenced). This would suggest that *S. microsporus* is a distinct species. Kurtzman and Fell (1991) examined the G + C contents of *S. ruineniae* (64.9%) and *S. coprophilus* (64.5%) and determined that DNA reassociation between the two strains was 63% which led them to suggest that the two strains represented varieties of the same species. As far as we are aware there have not been any G + C content or hybridization studies on the *S. microsporus* strain. The nucleotide sequence studies indicate that *Rhodosporidium fluviale* is related to these three species; the *S. ruineniae* strain differs from *R. fluviale* at nine base positions. Species separations of *R. ruineniae* and *R. fluviale* are confirmed by 0% DNA hybridization (Fell et al. 1992). The *S. microsporus* strain appears more closely related to *R. fluviale,* differing at five base positions; DNA hybridization studies between these two strains have not been reported.

87.4. *Sporidiobolus salmonicolor* Fell & Tallman (Fell & Statzell-Tallman 1981)

Anamorph: *Sporobolomyces salmonicolor* (Fischer & Brebeck) Kluyver & van Niel

Synonyms:

Rhodomyces kochii von Wettstein (1885)
Blastoderma salmonicolor Fischer & Brebeck (1894)
Monilia kochii (von Wettstein) Saccardo (1892)
Zygonema kochii (von Wettstein) de Mello (Froilano de Mello and Gonzaga Fernandes 1918)
Candida kochii (von Wettstein) Basgal (1931)
Sporobolomyces salmonicolor (Fischer & Brebeck) Kluyver & van Niel (1924)
Prosporobolomyces salmonicolor (Fischer & Brebeck) Novák & Zsolt (1961)
Aessosporon salmonicolor van der Walt (1970f)
Sporobolomyces odorus Derx (1930)
Sporobolomyces odorus Phaff (1970e)
Sporobolomyces photographus Biourge ex Ciferri & Redaelli var. *odorus* (Derx) Verona & Ciferri (1938)
Pseudomonilia rubicundula Okunuki (1931)
Sporobolomyces rubicundulus (Okunuki) Verona & Ciferri (1938)
Sporobolomyces hispanicus Peláez & Ramírez (1956a)
Prosporobolomyces hispanicus (Peláez & Ramírez) Novák & Zsolt (1961)
Sporobolomyces coralliformis Tubaki (1958)
Sporobolomyces salmonicolor (Fisher & Brebeck) Kluyver & van Niel var. *fischerii* Misra & Randhawa (1976)
Sporobolomyces philippovi Krasil'nikov (1933)
Sporidiobolus veronae Balloni, Florenzano & Materazzi (1974)

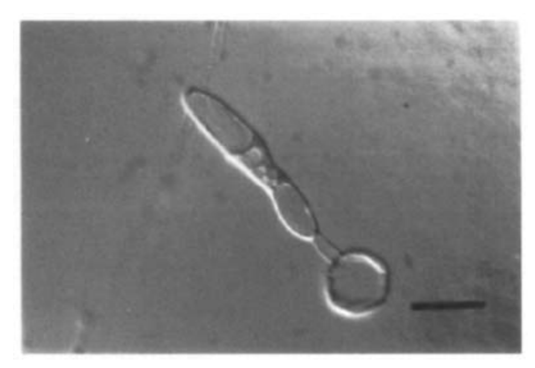


Fig. 355. *S. salmonicolor,* CBS 2630×CBS 5937. Opposite mating type cells were mixed on corn meal agar at 22°C; the resulting teliospores germinated within 18 weeks to a metabasidium connected to the teliospore by an aseptate basal cell. Bar = 10 μm.

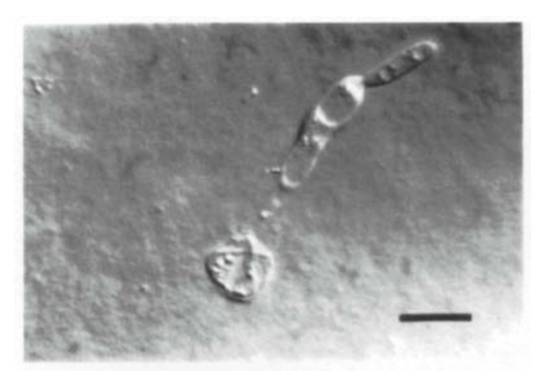


Fig. 356. *S. salmonicolor.* CBS 2630×CBS 5937. Opposite mating type cells were mixed on corn meal agar at 22°C; the resulting teliospores germinated within 18 weeks to a metabasidium connected to the teliospore by an aseptate basal cell. Bar = 10 μm.

Growth in 5% malt extract: After 7 days at 25°C, there may be a light to thick ring; when present the pellicle ranges from a thin film, or small islets, to thick and corrugated. Film and pellicle colors are off-white, light-salmon, yellowish-pink and peach. After one month the ring and pellicle have increased in size.

Growth on 5% malt agar: There is considerable strain variation in cell and colony morphology. After 7 days at 25°C, the cells are ovoid to elongate, (2–12)×(3–35) μm, single and in pairs; pseudo- and true hyphae may occur. Cells and hyphae may have sterigmata up to 50 μm or more in length. The streak cultures are shades of yellowish-pink, salmon and peach. The edges may be entire or heavily myceliated. The surface is smooth, powdery or deeply corrugated. The texture varies from pasty to tough. After one month, there is little change in the cell size, while the streak cultures become deeper in color.

Dalmau plate culture on corn meal agar: There is considerable strain variation, ranging from an absence of pseudo- and true hyphae to extensive growth of both types of hyphae.

Formation of ballistoconidia: Most strains form kidney-shaped, (3–5)×(5–10) μm, ballistoconidia on corn meal agar. Some strains produce ballistoconidia on malt agar.

Life cycle: The species is heterothallic. Conjugation of opposite mating types on corn meal agar at 22°C results in the formation of true mycelium with clamp connections at the septa. As noted by Bandoni et al. (1975) the mating reactions are not consistent, due possibly to such factors as types of media, age and condition of the strains. Two mating types have been recorded, indicating that the species is unifactorial with two alleles. The teliospores are spheroidal (10–14) μm or ovoidal (8–10)×(10–17) μm, terminal, intercalary and lateral on hyphal branches. The teliospores germinate after 18 weeks on corn meal agar at 22°C. The metabasidium is one-celled (5–7)×(13–47) μm and is borne on a connecting cell (1–3)×(3–13) μm (Fig. 355). One to two basidiospores form terminally on the metabasidium (Fig. 356). Hyphae may develop from the metabasidium. Endospores may be present in the metabasidium.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	s
D-Xylose	s	Succinate	v
L-Arabinose	v	Citrate	–
D-Arabinose	v	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	v
50% Glucose–yeast extract agar	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: 10 (Nakase and Suzuki 1986d).

Mol% G + C: 63.5–65.0 (BD: Storck et al. 1969); 62.0 (BD: Holzschu et al. 1981).

Cell hydrolyzates: Mannose, glucose, galactose, and fucose (von Arx and Weijman 1979); xylose absent (Suzuki and Nakase 1988a).

Origin of the strains studied: *Aessosporon salmonicolor* CBS 5937 (type strain, mating type *A1*); CBS 5937.3 (*A1*); *Sporobolomyces coralliformis* CBS 4209 (type), *A2*; *Sporobolomyces hispanicus* CBS 2873 (type), *A1*; *Sporobolomyces odorus* CBS 483 (type), *A2*; CBS 487 (*A1*); *Sporobolomyces salmonicolor* CBS 490 (type), *A1*; *S. salmonicolor* var. *fischerii* CBS 6832 (type), *A1*; CBS 1039 (*A1*); CBS 2630 (*A2*); strains UBC 949 (*A1*), UBC

950 (*A1*), UBC 958 (*A2*) from R.J. Bandoni; strains 5Y (*A1*) and 7Y (*A1*) from S.A. Meyer.

Complementary mating types: CBS 490, mating type *A1*, and allotype strain CBS 2630, mating type *A2*.

Type strain: CBS 490, type strain of *Sporobolomyces salmonicolor* (Kluyver & van Niel 1924), mating type *A1*.

Comments: Strains were isolated from sources as diverse as tanning fluid (Peláez Campomanes and Ramírez Gómez 1956a), seawater, atmosphere (Bandoni et al. 1971), mixed cultures (Kluyver and van Niel 1924); humans (Misra and Randhawa 1976, van der Walt 1970f) and plants (Tubaki 1958, Derx 1930).

Boekhout (1991a) reported a 75.8% DNA–DNA similarity between the type strains of *Sporobolomyces johnsonii* and *Sporidiobolus salmonicolor* and consequently placed *S. salmonicolor* into synonymy with *S. johnsonii.* LSU rRNA sequence analyses (Fell et al. 1992) of one mating strain of *S. salmonicolor* (UBC 949) and the type strain of *S. johnsonii* (CBS 5470) found that the two strains differed at 5 nucleotides (2% of the sequence examined). This result agrees with the conclusion that the two species are closely related, but questions the synonymy, and therefore we have retained the two taxa as separate species.

Comments on the genus

Nyland (1949) described a ballistoconidial yeast with clamp connections and thick-walled chlamydospores as the genus *Sporidiobolus,* a genus that represents the teleomorphic states of *Sporobolomyces.* The importance of this first observation of a sexual state for the basidiomycetous yeasts was probably overlooked as the genus was not mentioned in Lodder and Kreger-van Rij's (1952) review. With the discovery of a sexual state in *Rhodotorula* (Banno 1967), the significance of *Sporidiobolus* became apparent and Phaff (1970e) presented a detailed discussion of the genus and included a second species (*S. ruineniae*) in the genus. As currently viewed, the genus consists of the pigmented, teliospore- and ballistoconidia-forming members of the Sporidiales. There are four accepted species, which, based on nucleotide sequence analysis (Fell et al. 1992, 1995), are closely related. Species are differentiated by the method of teliospore formation (self-sporulation or heterothallic) and a limited number of assimilation tests (Table 71). Increased interest in the ballistoconidia-forming yeasts, as evidenced by the number of recently described species of *Sporobolomyces,* should result in the discovery of their teleomorphic states and consequently the addition of new species of *Sporidiobolus.*

88. *Sterigmatosporidium* Kraepelin & Schulze

A. Statzell-Tallman

Diagnosis of the genus

Growth on solid medium is white to cream-colored, either butyrous or rough. Both yeast and mycelial phases occur. Haploid yeast cells are polymorphic, ovoid to cylindrical, and reproduce by polar budding, usually on spiny denticles or thin sterigmata with the septa at the distal end. Pseudohyphae are present.

Heterothallic sexual reproduction occurs following conjugation of haploid cells of opposite mating types; dikaryotic hyphae with clamp connections form; clavate or lageniform cells develop with whorls of terminal dikaryotic basidia-like structures on clamps. Septal pores have not been examined. Self-sporulating strains occur which develop dikaryotic hyphae with clamp connections. Teliospores are absent. Monokaryotic haustorial branches form from subglobose cells on the hypha.

Fermentation is absent. D-Glucuronate and *myo*-inositol are assimilated. Starch-like compounds are synthesized. Diazonium blue B and urease reactions are positive. Coenzyme Q-10 is present. Xylose is present in whole-cell hydrolyzates.

Type species

Sterigmatosporidium polymorphum Kraepelin & Schulze

Species accepted

1. *Sterigmatosporidium polymorphum* Kraepelin & Schulze (1982)

Systematic discussion of the species

88.1. *Sterigmatosporidium polymorphum* Kraepelin & Schulze (1982)

Growth in 5% malt extract: After 3 days at 20°C, the cells are polymorphic, from oval, cylindrical, elongate to irregular and measure (1.1–3.7)×(1.6–10.1) μm. They occur singly, or bud on spiny denticles (Fig. 357) or sterigmata (Fig. 358) which are 0.5–3.7 μm long and 0.5 μm wide. New buds, which are ovoidal to spheroidal, separate from the distal end of the sterigmata or occasionally a sterigma breaks in the middle. A sediment is present. After one month, a sediment and sometimes islets or a pellicle are present.

Growth on 5% malt extract agar: After one month at 20°C, the streak colonies of the haploid and the dikaryon are white to cream-colored, raised, butyrous, smooth with buds forming on short stalks, or the colonies may be rugoid in which the buds form on long sterigmata (Fig. 358). The margin is entire, with the haploid strain fringed by pseudomycelium and the dikaryon fringed with both pseudomycelium and true mycelium with clamps at each septum.

Dalmau plate culture on corn meal agar: After one month at 20°C, pseudomycelium is formed by the haploid strains and dikaryotic strains. True mycelium is also formed by the dikaryotic strain.

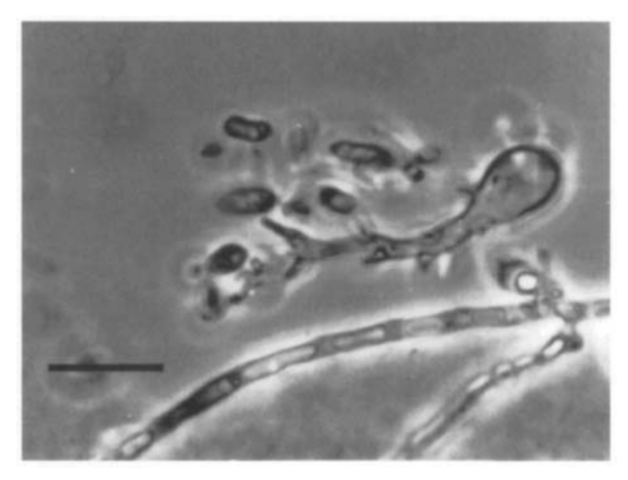


Fig. 357. *S. polymorphum*, CBS 8089×CBS 8090. Cell with spiny denticles formed on sympodial ramification seen in a mix of opposite mating types. Bar = 10 μm.

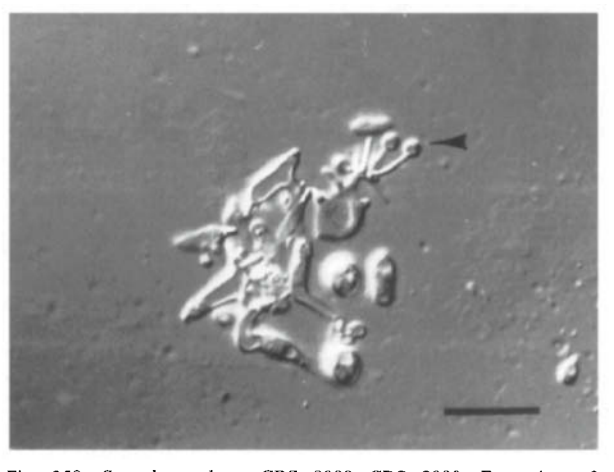


Fig. 358. *S. polymorphum*, CBS 8089×CBS 8090. Formation of blastoconidia (arrow) on long sterigmata. Bar = 25 μm.

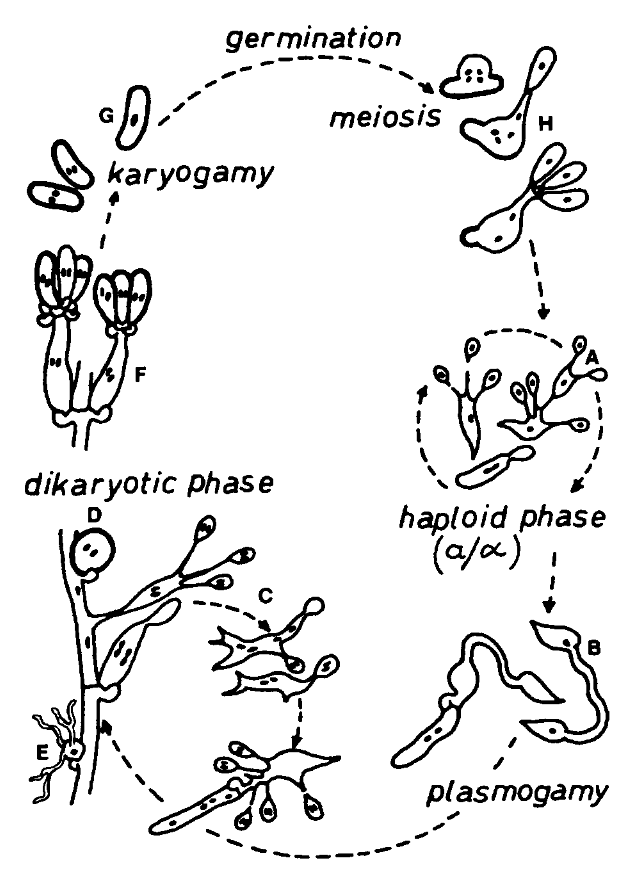


Fig. 359. *S. polymorphum.* Life cycle proposed by Kraepelin and Schulze (1982), pg. 479. (A) Haploid cells on long sterigmata. (B) Conjugation of haploid cells. (C) Dikaryotic conidia bud from dikaryotic hypha. (D) Chlamydospore-like cells with paired nuclei form with clamps on hypha. (E) Monokaryotic haustoria form from subglobose cells which develop on clamps from the dikaryotic hypha. (F) Refractive cells on whorl of clamp-like structures on lageniform sporogenous cell with basal clamp. (G) Refractive, dikaryotic basidia-like structures. (H) Haploid cells bud from basidia-like cells.

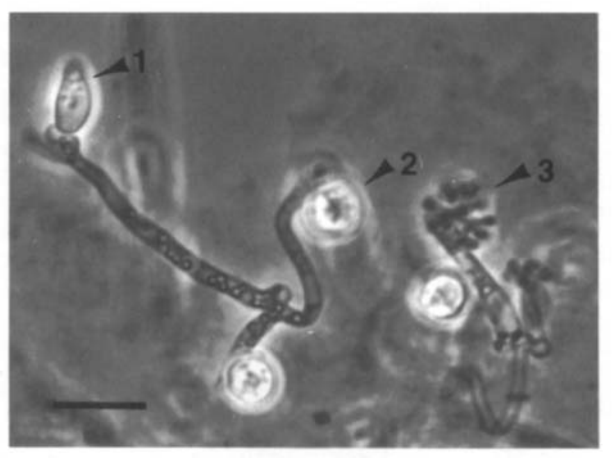


Fig. 360. *S. polymorphum.* CBS 8089×CBS 8090. Hypha with immature lageniform cell (1) on clamp and chlamydospores (2). Lageniform sporogenous cells on clamps with terminal whorls of clamps (3). Bar = 10 μm.

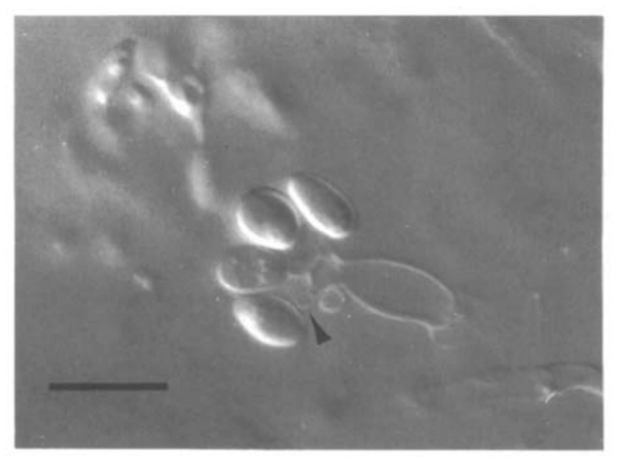


Fig. 361. *S. polymorphum.* CBS 8089×CBS 8090. Refractive cells formed on whorl of clamp-like or stalk-like structures (arrow) on lageniform sporogenous cell with basal clamp. Bar = 10 μm.

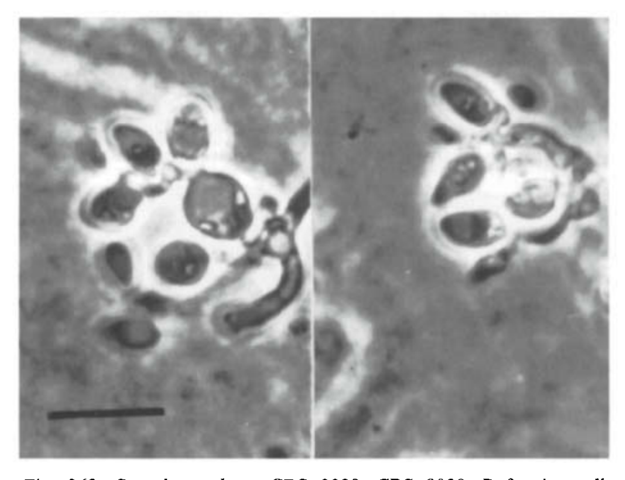


Fig. 362. *S. polymorphum.* CBS 8089×CBS 8090. Refractive cells formed on whorl of clamp-like or stalk-like structures on lageniform sporogenous cell with basal clamp. Bar = 10 μm.

Life cycle (from Kraepelin and Schulze 1982): Both haploid and dikaryotic self-sporulating strains may be isolated from nature. Mixing of opposite mating type haploid cells (Fig. 359A) on malt extract agar or corn meal agar results in conjugation (Fig. 359B) and the formation of hyphae with clamps at the septa. Lageniform to subglobose sporogenous cells form laterally or terminally with a clamp at the basal septum (Figs. 359F, 360).

These sporogenous cells develop whorls of an indeterminate number of cells, (2.9–3.8)×(4.8–6.7) μm, possibly sexual, each on a clamp-like or a stalk-like structure (Figs. 361, 362).

In the life cycle as presented by Kraepelin and Schulze (1982) the nuclei in these refractive, dikaryotic, basidia-like structures (Fig. 359G) are transversely paired. The authors suggested that karyogamy and meiosis occur in these cells, not in typical basidia, and that haploid cells of the two parental mating types bud from these sexual (?) cells (Fig. 359G,H). Self-sporulating strains may be derived from budding of dikaryotic conidia from

the dikaryotic mycelium (Fig. 359C). Spherical, refractive chlamydospore-like cells, (4.3–4.8)×(4.3–5.8) µm, with paired nuclei form with clamps on the hyphae (Figs. 359D, 360); germination of these cells is unknown. Monokaryotic haustoria form from subglobose cells which develop with clamps (Fig. 359E) from the dikaryotic hyphae.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	w
Maltose	+	Erythritol	s
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	+
Lactose	s	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Urease	+
Saccharate	s	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	w
50% Glucose-yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	s		

Co-Q: 10 (Yamada and Banno 1984b).
Mol% G+C: 51.96 (BD: Kurtzman 1990a).
Cell hydrolyzates: Xylose is present (Yamada et al. 1988a).

Origin of the strains studied: Gelatinous deposits on drenched pitwood planks in old ore mine, West Germany, Kraepelin, 1980.

Complementary mating types: CBS 8089 (Kraepelin strain Ra 18*a*) and CBS 8090 (Kraepelin Ra 18*α*).

Type strain: CBS 8088 (Kraepelin strain Ra 18), dikaryon.

Comments on the genus

The formation of blastoconidia on sterigmata initially linked *S. polymorphum* to *Sterigmatomyces* (Fell et al. 1984a). However there was little nucleotide complementarity (Kurtzman 1990a) between this species and the species accepted in the genus *Sterigmatomyces.* Subsequently, nucleotide sequence analyses (Guého et al. 1989, 1990, Fell et al. 1995), and electrophoretic comparisons of enzymes (Yamada et al. 1986a) indicated a close relationship with the genus *Fellomyces,* which clusters with the tremellaceous fungi. Further investigations on the life cycle and the relationship to other tremellaceous fungi are required.

89. *Tilletiaria* Bandoni & Johri

T. Boekhout

Diagnosis of the genus

Hyphae are septate, narrow, hyaline, and monokaryotic with retraction septa. Septal pores are primitive dolipores without parenthesomes. Ballistoconidia are allantoid and falcate, and formed on sterigmata. Chlamydospores are brown. Colonies are tough and grayish-brown.

Teliospores occur intercalarily or terminally, and are echinulate, brown, and germinate with 1–4-celled basidia. Clamp connections are absent.

Sugars are not fermented. Urease reaction is positive. Xylose is absent from whole-cell hydrolyzates. Major ubiquinone is Q-10.

Type species

Tilletiaria anomala Bandoni & Johri

Species accepted

1. *Tilletiaria anomala* Bandoni & Johri (1972)

Systematic discussion of the species

89.1. *Tilletiaria anomala* Bandoni & Johri (1972)

Growth on 5% malt extract agar: After 7 days at 25°C, hyphae are narrow, 1.0–3.5 µm wide, usually largely lysed with retraction septa, and with lateral sterigmata. Chlamydospores are subglobose to ellipsoidal, brown, (6.0–20.0)×(5.0–8.0) µm. Colonies are firm to tough, pruinose, dull dark grayish-brown, and with the margin entire or eroded.

Growth on the surface of assimilation media: Islets, a ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, hyphae are abundantly present. Aerobic growth is pale yellow-brown, ochraceous-brown to dark brown, firm, flat, irregular to pustulate, and the margin entire, eroded or crenulate.

Formation of ballistoconidia: Ballistoconidia are allantoid to falcate, (5.0–15.0)×(1.5–4.0) µm (Fig. 363).

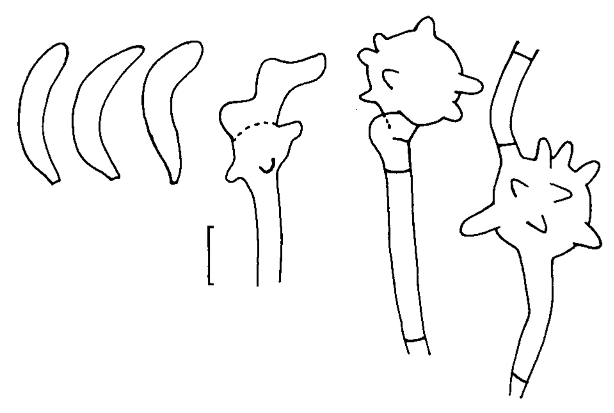


Fig. 363. *T. anomala*, CBS 436.72. Ballistoconidia and teliospores on morphology agar. Bar = 5 µm.

Formation of teliospores: Teliospores are subglobose, brown, echinulate, somewhat thick-walled, (6.0–12.5)×(6.0–12.5) µm, and with spines usually up to ca. 3.0 µm long (Fig. 363). Germination of teliospores occurs when transferred to water agar (1–3 days at 20–25°C), forming 1–4-celled basidia which form ballistospores (Bandoni and Johri 1972). The species is homothallic.

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	+	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	+
Urease	+	Growth at 35°C	–

Co-Q: 10, CBS 436.72 (Boekhout 1987, Boekhout et al. 1992a).

Mol% G+C: 62.9, CBS 436.72 (T_m: Boekhout et al. 1992b).

Origin of the strain studied: CBS 436.72 (ATCC 24039, UBC 951), decaying wood, R.J. Bandoni, Canada.

Type strain: UBC 951.

Comments on the genus

Tilletiaria is characterized by the presence of narrow hyphae without clamp connections, allantoid to falcate ballistoconidia, and brown, echinulate teliospores (Bandoni and Johri 1972, Boekhout 1991a). Prior to teliospore formation, outgrowths of two adjacent cells are formed near a septum, which after fusion, give rise to the teliospore.

Tilletiaria was tentatively placed in the Tilletiales, because of the presence of teliospores, basidiospores that are ballistospores, and from morphological, biochemical and ultrastructural similarities (Boekhout et al. 1992a).

The dark colonies hampered reading of the Diazonium blue B test, which is expected to be positive.

90. Tremelloid genera with yeast phases

Fibulobasidium Bandoni, *Holtermannia* Saccardo & Traverso, *Sirobasidium* de Lagerheim & Patouillard, *Tremella* Persoon, *Trimorphomyces* Bandoni & Oberwinkler

R.J. Bandoni and T. Boekhout

1. Introduction

The Tremellales and Filobasidiales are the only known taxa with both dolipore septa and yeast states. As noted in the introduction to heterobasidiomycetes, biochemical characteristics suggest a very close relationship of the two groups of fungi and maintaining the two as separate orders is primarily a matter of convenience here. The Tremellales in this sense includes only three families, the Tetragoniomycetaceae, Sirobasidiaceae, and Tremellaceae. The families Filobasidiaceae, Syzygosporaceae and Rhynchogastremaceae are presently placed in the Filobasidiales.

Although budding was first observed and illustrated in Tremellaceous fungi by Brefeld (1888) and by Möller (1895), later students mostly mentioned this trait as one of several methods of basidiospore germination, i.e., yeast states were not discussed. Dimorphism in heterobasidiomycetes, other than in smuts and their relatives, remained essentially unknown until the 1960's, when studies were carried out on species of *Tremella* Persoon (Bandoni 1961, 1963, Kobayasi and Tubaki 1965).

2. Classification

Taxa in two of the three families in the Tremellales, Tremellaceae s. str. (Bandoni 1984) and Sirobasidiaceae, have known yeast states. *Tetragoniomyces uliginosus* (Karsten) Oberwinkler & Bandoni, the only species in the Tetragoniomycetaceae, has deciduous basidia that function as disseminules and germinate directly (Oberwinkler and Bandoni 1981); the species is not considered further here. Basidia in the Sirobasidiaceae and Tremellaceae produce basidiospores, ballistospores and yeast states. All taxa in the order are thought to be mycoparasites, but the evidence for this largely rests upon association with specific fungi in nature, and experimental evidence for parasitism is lacking for most taxa. The Sirobasidiaceae and Tremellaceae are not sharply delimited morphologically, as noted below, and analyses of other types have not yet dealt with sufficient species to be of help at this level.

2.1. Life history

A life-history diagram typical of many Tremellales is shown in Fig. 364; it is based primarily upon studies of *Tremella mesenterica* Retzius:Fries (Bandoni 1963, 1965, Wong et al. 1985). Information from cultural studies of other taxa has been incorporated, including those of *T. globispora* Reid, *T. rhytidhysterii* Bezzera & Kimbrough (Bezerra and Kimbrough 1978), *Fibulobasidium inconspicuum* Bandoni (Bandoni 1979), and *Trimorphomyces papilionaceus* Bandoni & Oberwinkler (Oberwinkler & Bandoni 1983).

In culture, isolated basidiospores of heterothallic species give rise to yeast states, with mycelia typically developing only when compatible strains are paired. Pairing of suitable cells yields a rapid response to complementary pheromones; budding ceases, conjugation tubes develop and fuse, and the dikaryotic hyphal state is initiated. Under conditions suitable for fruiting, basidiome development commences soon after dikaryon initiation in *T. globispora* and *Sirobasidium magnum* (i.e., a long assimilative period is not required before basidiocarp production commences (Brough 1974, Flegel 1976). The basidiocarp typically is gelatinous and 2–4-celled basidia are produced in an amphigenous surface hymenium. Each basidial cell produces a fusiform, passively released spore (in Sirobasidiaceae) or a tubular epibasidium tipped by a sterigma and ballistospore (in most Tremellaceae). Basidiospores of both groups can germinate either by ballistospore production or by budding. Not infrequently, closely situated compatible basidiospores conjugate directly on culture media and, presumably, also in nature.

Differences in life histories and development are found in 1) dikaryotic hyphae, 2) basidiocarp features, 3) basidia, 4) basidiospore germination, 5) yeast states and conjugation, and 6) conidial production, as follows.

2.2. Dikaryotic hyphae

With few exceptions, dikaryotic hyphae in the Tremellales have clamp connections and characteristic haustorial branches. The latter, first recognized by Olive (1947) in *Tremella mycophaga* var. *obscura* Olive, and designated by him as haustoria, are known in many species of that genus. They have not been reported in *Sirobasidium* and are unknown in some species of Tremella. Each haustorial branch consists of a basal cell and filament; the latter contacts hyphal walls of associated fungi (Olive 1947, Bandoni 1961, Bezerra and Kimbrough 1978, Oberwinkler et al. 1984, Bandoni and Oberwinkler 1983, Metzler et al. 1989), the tip penetrating the wall. Bauer and Oberwinkler (1990a) showed direct, cytoplasm-to-cytoplasm connections between haustorial filaments of *Tetragoniomyces uliginosus* and its host, as well as in *Christiansenia pallida* Hauerslev (Syzygosporaceae) and its host (Bauer and Oberwinkler 1990b). Zugmaier et al. (1994) have found similar cytoplasmic connections between haustorial filaments of *Tremella mesenterica* and several associated basidiomycetes. Similar observations were made with *T. encephala* and *T. mycetophiloides*

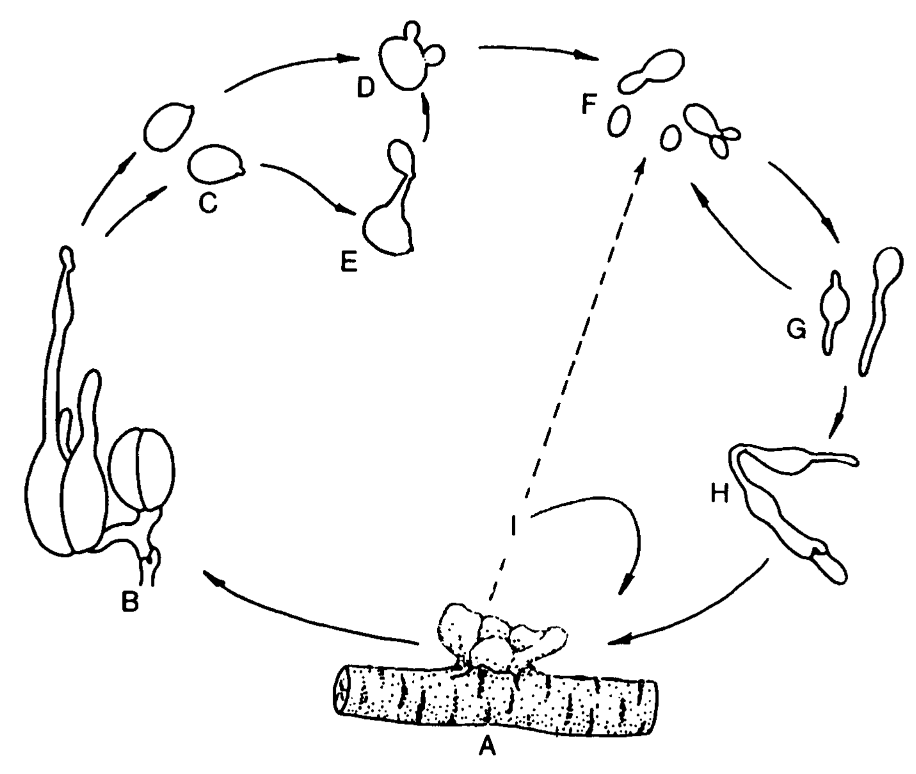


Fig. 364. Life cycle typical of the majority of Tremellales. (A) Basidiome on wood. (B) Basidia. (C) Basidiospores which can bud (D) or germinate by repetition (E). Budding initiates the yeast phase (F), which can continue indefinitely. If compatible cells are closely situated, complementary pheromones trigger tube development (G), and conjugation (H) occurs. Cells which fail to mate may revert to budding or produce conidia that bud. Conidia are also formed in many basidiomes (I). These include both dikaryotic conidia, which germinate by dikaryotic hyphal production, and haploid conidia which yield a yeast phase.

(as *T. mycophaga* Martin) with hyphae of *Stereum sanguineolentum* (Albertini & Schweinitz:Fries) Fries and *Aleurodiscus amorphus* (Persoon:Fries) Schröter, respectively. In *T. mycetophiloides* (Fig. 302D), which produces minute basidiomata on the host hymenium, the structures almost certainly function in absorption, as with haustoria in other parasites. Haustorial filaments in *Tremella mesenterica* can be found attached to hyphae of a variety of basidiomycetes, as noted by Zugmaier et al. (1994) and also to dematiaceous hyphae often growing in the basidiocarps. Contact commonly results in what appears to be a necrotrophic response, and the attack of many different types of hyphae by haustoria of this species may suggest a defensive function in addition to parasitism. In *Trimorphomyces papilionaceus* and *Tremella fuciformis* Berkeley, haploid yeast cells can also produce minute haustoria (Fig. 365) in the presence of suitable host hyphae (R.J. Bandoni, unpublished observation).

2.3. Basidiocarp features

Tremella penetrans (Hauerslev) Jülich, *T. mycophaga* Martin var. *obscura* Olive and several similar taxa grow as endoparasites of other basidiomycetes, e.g., dacrymycetaceous fungi and polypores. They do not have discrete basidiocarps, but produce basidia and often conidia in hymenia of their hosts. Basidiocarps in other species range from tuberculate structures under 1 mm in diameter in some lichen parasites to extensive masses reaching 20 cm in diameter in *Tremella brasiliensis* (Möller) Lloyd, *T. foliaceae* Persoon and others. A large size range also occurs in the Sirobasidiaceae. Basidiocarps in *Holtermannia* Saccardo & Traverso are composed of dense aggregates of erect simple or branched clavarioid structures.

Basidiocarps (Fig. 302) are entirely gelatinous in most species, but a tough fleshy core of host hyphae is present in those of *Tremella aurantia* Schweinitz:Fries, *T. encephala* Persoon (Bandoni 1961), and related taxa, and in some taxa overgrowing ascomycetous stromata, e.g., *T. moriformis* Smith & Sowerby. The structure is monomitic in species described to date; the usually amphigenous hymenial layer consists of basidia and, often, conidiogenous cells. Paraphysoids are present in a few species, e.g., *Tremella coalescens* Olive and in species closely related to *T. mesenterica.*

Two species, *T. fuciformis* and *T. aurantialba* Bandoni and Zang Mu, are cultivated commercially. *T. aurantialba* is grown in dual culture with a species of *Stereum* (Bandoni and Zang Mu 1990); basidiocarps contain zones of host hyphae as in *T. aurantia*. *T. fuciformis* (Fig. 365) also appears to be grown in mixed culture commercially, but the literature on the subject is unclear. Basidiocarps of this species are homogeneous and gelatinous; they are often associated with ascomata of *Hypoxylon* spp.

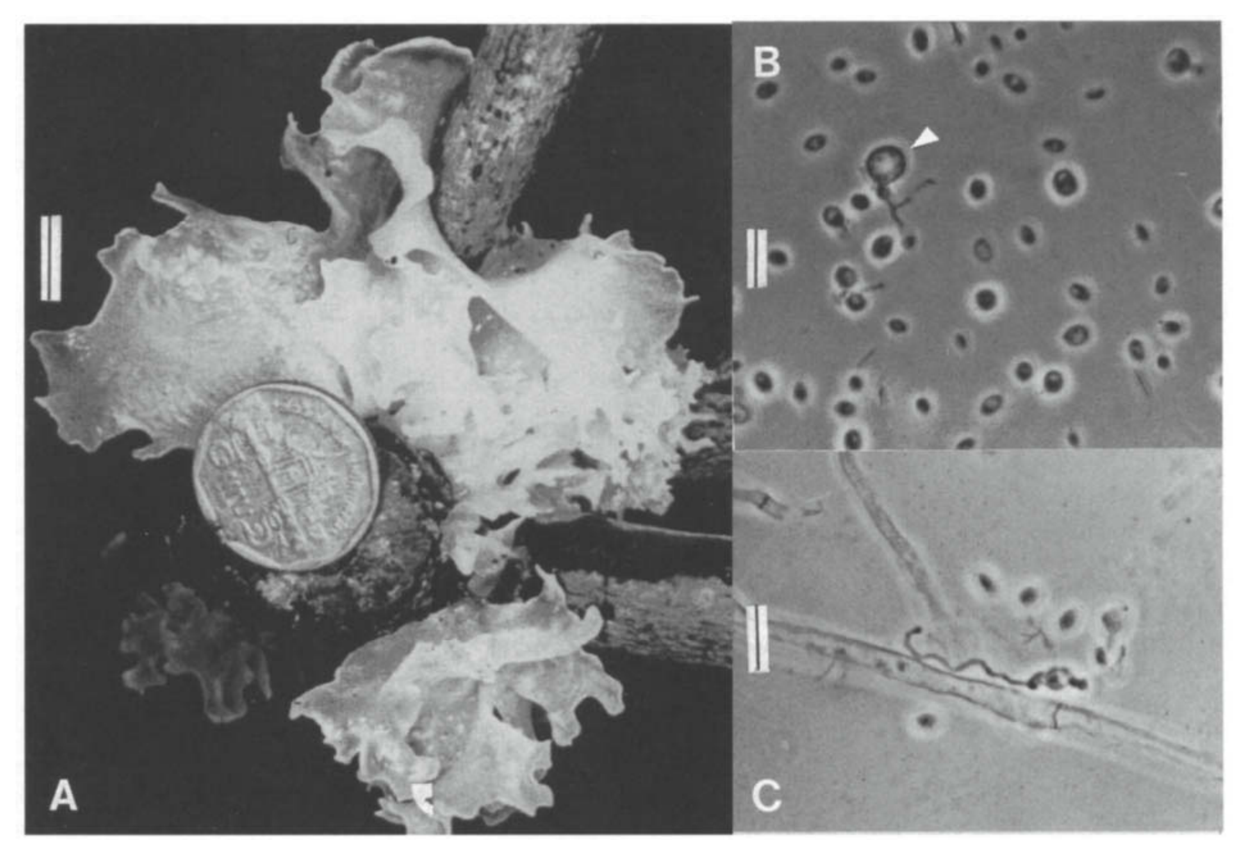


Fig. 365. *T. fuciformis* Berkeley. (A) Basidiome on dead branch. Bar = 10 mm. A-A & RJB 9832, Thailand. Photo courtesy of Dr. T.W. Flegel). (B) Mixed yeast strains of unknown compatibility growing with mycelium of *Hypoxylon stygium* (Lév.) Sacc., one cell (arrow) with haustorial branch. Bar = 10 µm. Cultures: UBC 7122-1(4), Hawaii; 7694-8, China. (C) Haustorial branch arising from short hypha (?haploid) out of focus below cell, the haustorial filament growing along a *Hypoxylon* hypha (UBC 7122-1, 7122-2, RJB, Thailand). *Hypoxylon stygium,* UBC 7668-C, RJB, China, used in both cultures shown in (B) and (C).

in nature. Culture studies suggest parasitism of these ascomycetes (R.J. Bandoni, unpublished observation). Xu Biru (1986) reported dependence upon an unnamed associate for fruiting in artificial culture. Cultivated basidiocarps of both species are dried and are widely sold as food; they are also used in traditional medicine in parts of Asia.

2.4. Basidia

Basidia in *Sirobasidium* occur in linear chains, which mature basipetally; those in *Fibulobasidium* Bandoni are in terminal clusters and are united to one another laterally. Here, basidia form successively, the first from a terminal cell subtended by a clamp connection; the second by direct inflation of that clamp connection, and the third by inflation of the basal clamp of the second, and so on. Basidia in the Tremellaceae are predominantly or all single and terminal, but scattered catenate ones occur in species of *Sirotrema* Bandoni and, less frequently, in *Tremella indecorata* Sommerfelt (Bandoni 1984).

Tremella simplex Jackson & Martin, *T. rhytidhysterii, Sirobasidium brefeldianum* Möller, and a few other taxa have 2-celled basidia, and 2-celled basidia sometimes occur in species characterized by 4-celled basidia. Basidial form, which is highly variable among species and within any single basidiome, ranges from globose to obovoid, pyriform, clavate, capitate, or subcylindric. The "septation pattern" (see Bandoni 1984, for an alternative concept) is regularly "cruciate" in most taxa, but is highly variable in others. Cylindric basidia with transverse septa are characteristic of *Phyllogloea tremelloidea* Lowy (F. Oberwinkler, personal communication) and *Phragmozenidium mycophilum* Oberwinkler & Schneller (Oberwinkler et al. 1990c). Basidia in *Sirobasidium* spp. are often fusiform and with 1 or 3 transverse to diagonal septa, i.e., they can be 2- or 4-celled, each cell budding off a fusiform primary basidiospore. Tremellaceous basidial cells develop tubular extensions (epibasidia), most of which are tipped by sterigmata and bear ballistospores. Passive release of epibasidia or basidiospores occurs either regularly or from time to time in some Tremellas, in *Sirotrema* spp., in *Bulleromyces albus* Boekhout & A. Fonseca (Boekhout et al. 1991a), and in the species of *Xenolachne* Rogers.

2.5. Basidiospore germination

The passively released basidiospores of *Sirobasidium* and *Fibulobasidium* spp., are probably homologues of epibasidia of most Tremellaceae; they are elongate, typically

fusiform, and germinate either by ballistospore formation or by budding. Basidiospores in the Tremellaceae are mostly ballistospores, but they also germinate either by producing another ballistospore (repetitive germination) or by budding. Details of basidiospore budding differ among the species, and there can also be differences among spores of a single basidiome. Budding can occur directly, with the buds falling free at maturity; in *T. mesenterica,* primary buds remain attached to the basidiospore (Fig. 290) and produce buds that are freed (as illustrated, e.g., by Ingold 1982a,b, 1985, Bandoni 1987). These two types may be combined with another, in which a short broad tube is produced and buds or sessile budding cells develop at its apex.

2.6. Yeast states and conjugation

Haploid yeast states in species of *Tremella* (Figs. 290, 365) were noted almost 30 years ago to resemble asporogenous yeasts classified in the genus *Cryptococcus* (Kobayasi and Tubaki 1965, Slodki et al. 1966). More recent studies of coenzyme Q (Yamada and Kondo 1973, Yamada et al. 1983) and carbohydrate composition also show similarities between tremelloid and cryptococcoid yeasts. Comparison of the above two characteristics, as well as septal pore ultrastructure, ballistospores, and haustoria by Boekhout et al. (1992b) suggests a close relationship of *Bullera* Derx, *Bulleromyces* Boekhout & Fonseca, *Kockovaella* Nakase et al., and *Itersonilia* Derx to the Tremellales. The classification of *Bulleromyces* and the *Bullera* anamorphs definitely should be in the Tremellales, but the relationship of *Kockovaella* and *Itersonilia* species are not yet firmly established. Similarly, a definite link between *Tremella* and *Cryptococcus* species has not been established, i.e., no *Cryptococcus* strains have been found to yield a *Tremella*-like teleomorph. Although Baharaeen and Vishniac (1984) suggested that 25S ribosomal RNA homology data did not support a close relationship, studies by Guého et al. (1993), Fell et al. (1992, 1995), and Swann and Taylor (1993) have found otherwise. *Tremella* yeasts examined to date all have the coenzyme Q-10 system, as do closely related species of *Filobasidium* (Yamada et al. 1983) and some species placed in *Cryptococcus* (Yamada and Kondo 1973). The Q-10 system does not appear to be definitive, however, in that it is widespread in hemiascomycetes as well as other heterobasidiomycetes (Yamada et al. 1982, 1983).

An unusual and striking feature is the occurrence of a morphologically distinct dikaryotic yeast phase in *Trimorphomyces papilionaceus* (Oberwinkler and Bandoni 1983). This phase is initiated following conjugation of haploid yeast cells, or it can develop at germination of dikaryotic conidia produced on conidiophores. The dikaryotic yeast phase is usually characterized by colonies of H-shaped, binucleate cells, that are morphologically identical to the conidia. Dikaryotic cells superficially similar to the haploid ones are produced in rare strains. The cells produce pairs of closely situated "twin" buds synchronously, and conjugation occurs between the two individuals of a pair before their release. A similar development in species of *Syzygospora* Martin involves conjugation of adjacent buds from paired (separate) conidiogenous cells, but no dikaryotic yeast states are known in that group.

In heterothallic species of *Tremella, Sirobasidium, Holtermannia, Fibulobasidium,* and *Trimorphomyces* examined to date, isolated basidiospores germinate by budding (Fig. 290), and this then continues indefinitely under laboratory conditions. Mating is initiated by an allelic pair, the A's controlling production of complementary pheromones; independent alleles, the B's (multiple alleles at one locus) determine dikaryon viability (Bandoni 1963, 1965, Brough 1974, Flegel 1976, Wong et al. 1985, Fox and Wong 1990, Okuda et al. 1981). To be compatible, pairs must be heterozygous for both sets of alleles, the first to initiate the conjugation process, and the second in order to yield a dikaryotic mycelial phase. Pairing strains heterozygous for A's and homozygous for B's (common B) often results in plasmogamy, yielding cells which are usually vesiculose and abortive. The pheromones produced by *Tremella mesenterica* and *T. brasiliensis* are peptides (Reid 1974, Sakagami et al. 1978a,b, 1979, Ishibashi et al. 1983a,b) similar to those in species of *Rhodotorula* Harrison. When compatible cells are mixed, short tubes can often be seen after 4 hours; extensive tube development and short dikaryotic hyphae are visible within 12 hours. Conjugation tubes in *Trimorphomyces papilionaceus* are extremely inconspicuous; in the absence of host hyphae in culture, conjugation can be detected by the presence of the dikaryotic H-shaped yeast cells. Canadian isolates of the species show strong sexual agglutination (R.J. Bandoni, unpublished observation), but this is absent in Japanese strains.

Secondary homothallism, in which binucleate basidiospores give rise directly to dikaryotic hyphae, is known in *Sirobasidium magnum* and *Tremella rhytidhysterii.* Many basidia in young basidiomes of *S. magnum* are two-celled, and the basidiospores from such basidia produce fertile mycelia directly (R.J. Bandoni, unpublished observation). The two-celled basidia of *T. rhytidhysterii* produce basidiospores that give rise to dikaryotic mycelia; the latter sometimes dedikaryotize to yield haploid yeast states (Bezerra and Kimbrough 1978). Homothallism has also been found in stocks of *T. fuciformis* from Hawaii and Panama, although most isolates of the species are heterothallic (Fox and Wong 1990). Many strains in this species also have limited development of haploid hyphae with false clamps and occasional haustorial branches.

2.7. Conidial production

Dikaryotic conidia are produced on growing hyphae of *Tremella mesenterica* in culture, as is the production of scattered pockets of haploid budding cells in this species and others (Brough 1974, Bezerra and Kimbrough 1978).

In basidiocarps, conidia may develop before basidia, or concurrently with them in the hymenium of *T. mesenterica* (Fig. 300). They also occur concurrently with basidia in *T. mycetophiloides* Kobayasi, *T. australiensis* Lloyd, and *Trimorphomyces papilionaceus* (Fig. 304). Hymenial conidia appear to be mainly blastic, but combinations of arthric and blastic development are characteristic of some taxa. Frequent pockets of budding cells within the basidiome tissue, sometimes in the absence of visible basidia and spores, suggests occasional dedikaryotization similar to that occurring in culture.

3. Descriptions and keys to representative yeast states

The number of species known in the families Sirobasidiaceae and Tremellaceae exceeds 150, but many species remain to be described in both temperate and tropical regions. Biochemical analyses and morphological studies indicate that *Tremella,* the largest genus, is heterogeneous. However, very few taxa have been examined carefully by either approach. Among the undescribed taxa are many growing on lichens (Diederich 1986, 1996, and personal communication). In this treatment, yeast states of 17 common temperate and tropical species are described. For convenience, haploid yeast states of the various species are referred to by their teleomorph names. The same species are also mentioned in the keys to similar anamorphic yeast genera (e.g., *Cryptococcus, Bullera,* etc.). Detailed descriptions of the teleomorphs are available in taxonomic works dealing with these fungi, e.g., Bandoni 1958, Bandoni and Oberwinkler 1983, Bourdot and Galzin 1928, Kobayasi 1937, 1939, Martin 1952, Pilat 1957, Torkelsen 1968, Raitvir 1967, Lowy 1971, 1980, Wojewoda 1981, and in references cited in these works.

4. Key to species with yeast phases

1.	a *myo*-Inositol assimilated → 2		
	b *myo*-Inositol not assimilated → 14		
2(1).	a Inulin assimilated	*Bulleromyces albus*:	p. 641
	b Inulin not assimilated → 3		
3(2).	a Sucrose assimilated → 4		
	b Sucrose not assimilated → 12		
4(3).	a Ethanol assimilated → 5		
	b Ethanol not assimilated → 11		
5(4).	a D-Glucosamine assimilated (may be delayed) → 6		
	b D-Glucosamine not assimilated → 9		
6(5).	a Nitrite assimilated, erythritol delayed	*Fibulobasidium inconspicuum*:	p. 710
	b Nitrite and erythritol not assimilated → 7 *(Tremella aurantia, T. encephala, T. indecorata)*		
7(6).	a Growth at 30°C → 8		
	b Growth absent at 30°C	*Tremella aurantia*:	p. 712
		30°C negative strains of *T. indecorata*:	p. 716
8(7).	a Growth at 37°C	*Tremella encephala*:	p. 714
	b Growth absent at 37°C	*Tremella indecorata*:	p. 716
		37°C negative strains of *T. encephala*:	p. 714
9(5).	a Raffinose assimilated → 10		
	b Raffinose not assimilated	*Filobasidium inconspicuum*:	p. 710
10(9).	a Lactose assimilated	*Sirobasidium intermedium*:	p. 710
	b Lactose not assimilated	*Holtermannia corniformis*:	p. 711
11(4).	a Melibiose and soluble starch assimilated	*Trimorphomyces papilionaceus*:	p. 717
	b Melibiose and soluble starch not assimilated	*Tremella moriformis*:	p. 717
12(3).	a Lactose assimilation delayed	*Sirobasidium magnum*:	p. 711
	b Lactose not assimilated → 13		
13(12).	a Soluble starch assimilated	*Holtermannia corniformis*:	p. 711
	b Soluble starch not assimilated	*Tremella foliacea*:	p. 714
14(1).	a 2-Keto-D-gluconate assimilated → 15		
	b 2-Keto-D-gluconate not assimilated → 17		
15(14).	a Raffinose assimilated → 16		
	b Raffinose not assimilated	*Tremella globispora*:	p. 715
16(15).	a L-Rhamnose assimilated	*Trimorphomyces papilionaceus*:	p. 717
	b L-Rhamnose not assimilated	*Tremella fuciformis*:	p. 715
17(14).	a Melezitose assimilated → 18		
	b Melezitose not assimilated → 19		
18(17).	a Galactose assimilated	*Tremella cinnabarina*:	p. 713
		2-keto-D-gluconate negative strains of *T. fuciformis*:	p. 715
	b Galactose not assimilated	*Tremella coalescens*:	p. 713
19(17).	a Galactose assimilated, maltose delayed	*Tremella brasiliensis*:	p. 712
	b Galactose and maltose not assimilated	*Tremella mesenterica*:	p. 716

5. Taxa described

5.1. Sirobasidiaceae

1. *Fibulobasidium inconspicuum* Bandoni (1979)
2. *Sirobasidium intermedium* Kundalkar & Patil (1986)
3. *Sirobasidium magnum* Boedijn (1934)

5.2. Tremellaceae

4. *Bulleromyces albus* Boekhout & Fonseca (1991)
5. *Holtermannia corniformis* Kobayasi (1937)
6. *Tremella aurantia* Schweinitz:Fries (1822)
7. *Tremella brasiliensis* (Möller) Lloyd (1922)
8. *Tremella cinnabarina* (Montagne) Patouillard (1900)
9. *Tremella coalescens* Olive (1951)
10. *Tremella encephala* Persoon (1822)
11. *Tremella foliacea* (Persoon:S.F. Gray) Persoon (1822)
12. *Tremella fuciformis* Berkeley (1856)
13. *Tremella globispora* Reid (1970)
14. *Tremella indecorata* Sommerfelt (1826)
15. *Tremella mesenterica* Retzius:Fries (1822)
16. *Tremella moriformis* Smith:Berkeley (1856)
17. *Trimorphomyces papilionaceus* Bandoni & Oberwinkler (1983)

6. Systematic discussion of the species

90.1. *Fibulobasidium inconspicuum* Bandoni (1979)

Growth on malt agar: After 3 days at 25°C, the cells are subglobose to globose, (2.5–5.5)×(2–5.5) µm, and single or in pairs. After one month at 20°C, the streak culture is gray-brownish-yellow, pasty, partly wrinkled and partly smooth with a lobate edge.

Dalmau plate culture on potato agar: Pseudohyphae and hyphae are absent.

Formation of ballistospores: Ballistospores are fusiform to ellipsoidal, and measure (2–8)×(1.5–3.5) µm.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	l
Maltose	+	Erythritol	s
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	s	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	s	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	w
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	+	Inositol	+
D-Ribose	s	Hexadecane	n
L-Rhamnose	s	Nitrate	v
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	–
Nitrite	+		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 6963, CBS 7672, CBS 7679, CBS 7680, CBS 8237, CBS 8238, all from oak (*Quercus virginiana*), USA.

90.2. *Sirobasidium intermedium* Kundalkar & Patil (1986)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal to subglobose, (4.0–7.5)×(2.3–7.0) µm, and single. Budding is polar or occasionally multilateral, sessile or on short denticles, and with sympodial proliferation. Colonies are yellowish to yellowish-cream, mucoid, smooth, shiny, pale and with the margin entire.

Growth on the surface of assimilation media (glucose): Some flocks, a fragile ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 5 days at 25°C, primitive pseudohyphae may be present.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	l	Ethanol	+
Sucrose	+	Glycerol	l
Maltose	+	Erythritol	s
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	s
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	s
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	s
Starch formation	+	Growth at 30°C	s
Urease	+	Growth at 37°C	–
Nitrite	–		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: CBS 7805, from *Eutypella leprosa* on elm (*Ulmus* sp.), Devon, UK, also reported from India (Kundalkar and Patil 1986).

90.3. *Sirobasidium magnum* Boedijn (1934)

Growth on malt agar: After 3 days at 25°C, the cells are globose to subglobose, (2–4.5)×(2.5–6) µm, and single or in pairs. After one month at 20°C, the colonies are yellowish or yellowish-white, smooth or with a finely reticulate border, shiny or dull in the middle of the streak, and with a smooth or undulating edge.

Dalmau plate culture on potato agar: Pseudohyphae and hyphae are absent.

Formation of ballistospores: Ballistospores on corn meal agar are spherical, ovoidal or apiculate, and measure (4–9)×(3.5–5) µm.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	l	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	s
Maltose	v	Erythritol	s
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	l
Lactose	s	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	l
Melezitose	l	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	l
D-Xylose	+	Succinate	l
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	s	Hexadecane	n
L-Rhamnose	s	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	–
Nitrite	–		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 6803, CBS 6804, CBS 6805, CBS 6964, on decaying wood and oak (*Quercus* sp.), USA, Japan and Philippines.

90.4. *Bulleromyces albus* Boekhout & A. Fonseca (1991)

See *Bulleromyces albus*: p. 641

90.5. *Holtermannia corniformis* Kobayasi (1937)

Growth on malt agar: After 3 days at 25°C, the cells are short ellipsoidal to elongate, (5–15)×(2.5–5) µm, and short hyphal elements may occur. Budding is polar, and sometimes on a broad base. After one month at 20°C, the colonies are yellowish-white, smooth, shiny and mucoid.

Dalmau plate culture on potato agar: Pseudohyphae and extensive hyphae are absent, but occasionally a few hyphal cells, sometimes with septa, are present.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	v	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	s
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	l
Soluble starch	+	DL-Lactate	l
D-Xylose	+	Succinate	s
L-Arabinose	l	Citrate	s
D-Arabinose	s	Inositol	+
D-Ribose	v	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	v
Nitrite	–		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 6979, CBS 7088, CBS 7675, CBS 7676, from dead wood, Japan.

90.6. *Tremella aurantia* Schweinitz:Fries (1822)

Growth on malt agar: After 3 days at 25°C, the cells are ellipsoidal, (3–5.5)×(2.5–4.5) µm, and single or in pairs. Budding occurs on a rather broad base. After one month at 20°C, the colonies are yellowish-brown, smooth, shiny, and slimy.

Slide culture on potato agar: Pseudohyphae and hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	w/–	Diazonium blue B	+
Starch formation	+	Growth at 25°C	+
Urease	+	Growth at 30°C	–

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strains studied: CBS 6965, CBS 8203, CBS 8213, on angiosperm wood, widely distributed, Canada.

Comments: The yeast stage of *Tremella aurantia* is very similar to that of *T. encephala* and *T. indecorata.* With the set of identification tests currently used, these species can hardly be differentiated. However, *T. aurantia* does not assimilate melibiose and lacks growth at 30°C, whereas the other species show a variable or a slow response.

90.7. *Tremella brasiliensis* (Möller) Lloyd (1922)

Growth on malt agar: After 3 days at 25°C, the cells are ellipsoidal, (2.5–5.5)×(1.5–4.0) µm, and single or in pairs. After one month at 20°C, the colonies are whitish-brown, smooth, shiny, somewhat slimy, and with a smooth edge.

Slide culture on potato agar: Pseudohyphae and hyphae are absent, but cells may adhere in short chains.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	s	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	s	Erythritol	l
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	s	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	l
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	s	Inositol	–
D-Ribose	l	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	–
50% Glucose	–	Diazonium blue B	+
Starch formation	+	Growth at 25°C	+
Urease	+	Growth at 30°C	–

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strains studied: CBS 6966, CBS 8209, CBS 8212, CBS 8231, from Costa Rica.

90.8. *Tremella cinnabarina* (Montagne) Patouillard (1900)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are subglobose to ellipsoidal, (5.0–8.0)×(3.0–8.0) μm, and single. Budding is polar or occasionally multipolar, usually on a short denticle, and with sympodial proliferation. Colonies are grayish-cream, shiny, smooth, somewhat mucoid and with the margin entire.

Growth on the surface of assimilation media (glucose): A fragile ring and a sediment are formed.

Dalmau plate culture on morphology agar: Pseudohyphae and hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Diazonium blue B	s
50% Glucose	–	Growth at 25°C	+
Starch formation	v	Growth at 30°C	+
Urease	+	Growth at 37°C	–
Nitrite	–		

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strain studied: CBS 8234, origin unknown.

90.9. *Tremella coalescens* Olive (1951)

Growth on malt agar: After 3 days at 25°C, the cells are ellipsoidal, (3–6)×(1.5–3.5) μm, and single or in pairs. After one month at 20°C, the colonies are whitish-brown, smooth, shiny, somewhat slimy and with a smooth edge.

Slide culture on potato agar: Pseudohyphae and true hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	s	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	s
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	s	Succinate	s
L-Arabinose	–	Citrate	s
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	–
50% Glucose	–	Diazonium blue B	s
Starch formation	+	Growth at 25°C	+
Urease	+	Growth at 30°C	–

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strain studied: CBS 6967, from an angiosperm in a swamp, USA.

90.10. *Tremella encephala* Persoon (1822)

Growth on malt agar: After 3 days at 25°C, the cells are globose, subglobose to short ellipsoidal, (2.5–9)×(1.8–7) μm, and single or in pairs. After one month at 20°C, the colonies are yellowish-brown, smooth, shiny, and slimy.

Slide culture on potato agar: Pseudohyphae and true hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	l
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	s
Lactose	s	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	s	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	s
Urease	+	Growth at 37°C	v
Nitrite	–		

Co-Q: Not determined.

Mol% G + C: Not determined.

Origin of the strains studied: CBS 6968, CBS 8207, CBS 8217, CBS 8218, CBS 8220, from conifer wood (rarely on angiosperms, cosmopolitan in temperate conifer forests), Canada.

Comments: The yeast stage of this species is difficult to differentiate from the yeast stages of *T. aurantia* and *T. indecorata* using the present set of characteristics (see also under *T. aurantia* and *T. indecorata*.

90.11. *Tremella foliacea* (Persoon:S.F. Gray) Persoon (1822)

Growth on malt agar: After 3 days at 25°C, the cells are globose, subglobose to short ellipsoidal, (3–8.5)×(2–8) μm, and single or in pairs. After one month at 20°C, the colonies are whitish-brown, smooth, shiny, somewhat slimy, and with an entire margin.

Slide culture on potato agar: Pseudohyphae and hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	l	Erythritol	v
Cellobiose	l	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	v	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	s
Urease	+	Growth at 37°C	–
Nitrite	l		

Co-Q: Not determined.

Mol% G + C: Not determined.

Origin of the strains studied: CBS 6969, CBS 7215, CBS 8208, CBS 8210, CBS 8227, on wood of deciduous trees (*Acer macrophyllum*) and conifers (*Pinus mugo*), Canada and Germany.

90.12. *Tremella fuciformis* Berkeley (1856)

Growth on malt agar: After 3 days at 25°C, the cells are globose, subglobose to ellipsoidal, (2.5–7)×(2–4.5) µm, and single or in pairs. After one month at 20°C, the colonies are yellowish-brown, smooth, shiny and slimy.

Slide culture on potato agar: Pseudohyphae and hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	l
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	l	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	l	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	–
Nitrite	–		

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strains studied: CBS 6970, CBS 6971, CBS 8225, CBS 8226, from, e.g., the fruiting bodies of commercially grown strains, Taiwan and Japan; pantropical and extending onto warm temperate areas.

90.13. *Tremella globispora*[1] Reid (1970)

Growth on malt agar: After 3 days at 25°C, budding yeast cells and true hyphae are present. The yeast cells are globose to ellipsoidal, (4–8)×(3–5.5) µm, and single or in pairs. The hyphae have a diameter of ca. 2 µm. After one month at 20°C, the colonies are yellowish-white to brown, smooth, shiny, and very slimy.

Slide culture on potato agar: Pseudohyphae and true hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	s
Cellobiose	s	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	s	Citrate	+
D-Arabinose	s	Inositol	–
D-Ribose	s	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	s	Nitrite	–
50% Glucose	–	Diazonium blue B	+
Starch formation	v	Growth at 25°C	+
Urease	+	Growth at 30°C	–

Co-Q: Not determined.

Mol% G+C: Not determined.

Origin of the strain studied: CBS 6972, from *Diaporthe* on *Cornus nuttalli*, Canada.

Comments: In the original description, this name was incorrectly spelled as *T. globospora*.

[1] The original spelling of the species name as *globospora* has been treated as an orthographic error.

90.14. *Tremella indecorata* Sommerfelt (1826)

Growth on malt agar: After 2 days at 25°C, the cells are globose, subglobose to elliopsoidal, (2.5–8)×(2–4.5) µm, and single or in pairs. After one month at 20°C, the colonies are yellowish-brown, smooth, shiny, and very slimy.

Slide culture on potato agar: Pseudohyphae and true hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	l
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	l
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	–
Nitrite	–		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 6976, CBS 8232, from willow (*Salix* sp.), Canada.

Comments: The yeast stage of this species is difficult to differentiate from the yeast phases of *T. aurantia* and *T. encephala*. However, it may be differentiated from the former by variable growth responses with melibiose and at 30°C, and from the latter by lack of growth at 37°C.

90.15. *Tremella mesenterica* Retzius:Fries (1822)

Growth on malt agar: After 3 days at 25°C, the cells are globose to subglobose or short ellipsoidal, sometimes cylindrical, (2.5–6)×(2–5) µm, occasionally up to 16 µm long, single, and in pairs or in short chains. After one month at 20°C, the colonies are yellowish-white to brown, smooth, shiny, and somewhat slimy.

Slide culture on potato agar: Pseudohyphae and true hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	l	Ethanol	l
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	l
Cellobiose	l	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	s	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	l
Inulin	–	D-Gluconate	l
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	l
L-Arabinose	–	Citrate	l
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	–
Nitrite	–		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 6973, CBS 6974, CBS 6975, CBS 8211, CBS 8214, from dead wood of angiosperms, e.g., alder (*Alnus* sp.), Canada, Costa Rica.

90.16. *Tremella moriformis* Smith:Berkeley (Berkeley 1860)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal, (4.5–9.0)×(3.0–6.0) μm, and single; larger cells, (9.0–16.0)×(4.0–6.5) μm, occur as well, and may form short branched or unbranched chains. Budding is polar, on short denticles and sometimes with sympodial proliferation. Colonies are whitish, shiny, smooth, mucoid and with the margin entire.

Growth on assimilation media (glucose): A thin film and ring, and a sediment are formed.

Dalmau plate culture on morphology agar: After 5 days at 25°C, filaments are present, which may form primitive pseudohyphae.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	s	Ribitol	s
Trehalose	+	Galactitol	s
Lactose	l	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	s	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	l
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	s	Inositol	w
D-Ribose	s	Hexadecane	n
L-Rhamnose	s	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Diazonium blue B	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	s
Urease	+	Growth at 37°C	–
Nitrite	l		

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strain studied: CBS 7810, on a dead branch of an alder (*Alnus rubra*), Canada.

90.17. *Trimorphomyces papilionaceus* Bandoni & Oberwinkler (1983)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are subglobose, ellipsoidal, somewhat triangular, oblong or subcylindric, (4.5–7)×(3–6) μm, and single. Budding is polar, but sometimes multipolar, usually on a short denticle, and the cells may adhere in short chains. Cells up to 10.0 μm long occur, which bud on a broad base, leaving distinct scars. Colonies are pale pinkish-yellow to pale pinkish-brown, smooth, shiny, butyrous, and with the margin entire. Colonies of the dikaryotic yeasts are superficially similar, but the cells are H-shaped to irregularly butterfly-shaped; budding is usually polar, twin buds developing synchronously on closely situated points; conjugation occurs between these paired cells early in their development, and they are released as H-shaped cells.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: Pseudohyphae and hyphae are absent.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	–
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	s
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	l
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	+	Inositol	v
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	–	Diazonium blue B	+
Starch formation	v	Growth at 25°C	+
Urease	+	Growth at 30°C	–

Co-Q: Not determined.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 443.92, CBS 445.92, CBS 200.94, on the stem of *Arundinaria*-like cane, on culms of *Sasa* sp. and on the stem of bamboo, Japan and Canada.

91. *Xanthophyllomyces* Golubev

W.I. Golubev

Diagnosis of the genus

Cells are encapsulated and reproduce by enteroblastic budding. True mycelium is absent; chlamydospores may be present. Slender cylindrical holobasidia arise mainly after conjugation between a cell and its bud. Basidiospores are produced terminally on minute pegs. They are not forcibly discharged and germinate by budding. Carotenoid pigments are synthesized, which give cultures an orange to salmon-red color. Xylose and rhamnose are present in extracellular polysaccharides. The urease and diazonium blue B tests are positive. Glucuronate assimilation is variable.

[1]Fermentation is present. *myo*-Inositol and nitrate are not assimilated. Starch-like compounds are produced. Coenzyme Q-10 is formed.

Type species

Xanthophyllomyces dendrorhous Golubev

Species accepted

1. *Xanthophyllomyces dendrorhous* Golubev (1995)

Systematic discussion of the species

91.1. *Xanthophyllomyces dendrorhous* Golubev (1995)

Anamorph: *Phaffia rhodozyma* Miller, Yoneyama & Soneda[2]
Synonyms:
?*Rhodomyces dendrorhous* Ludwig (1891)
Rhodozyma montanae Phaff, Miller, Yoneyama & Soneda (1972) nom. nud.
Phaffia rhodozyma Miller, Yoneyama & Soneda (1976b)
Cryptococcus rhodozyma (Miller, Yoneyama & Soneda) Weijman, Rodrigues de Miranda & van der Walt (1988)

Growth in glucose–yeast extract–peptone water: After 3 days at 18°C, the cells are spheroidal to ovoidal (width/length ratios are 1.0 to 2.5, av. 1.4), 3.5–10.0×5.0–13.5 µm, (av. 6.0×8.5 µm) with small capsules, and single, in pairs, or occasionally in short chains. A very thin creeping pellicle may be present. After 1 month, there is a sediment, a ring, and sometimes islets.

Growth on Difco yeast morphology agar: After 1 month at 18°C, the streak culture is orange to salmon-red, nearly smooth, glossy to semi-dull, soft; the margin is entire or undulating. Spheroidal chlamydospores with refractile granules may be formed.

Slide cultures on corn meal agar: After 10 days at 18°C, a rudimentary pseudomycelium may occur. No true mycelium is formed. Ballistospores are not observed.

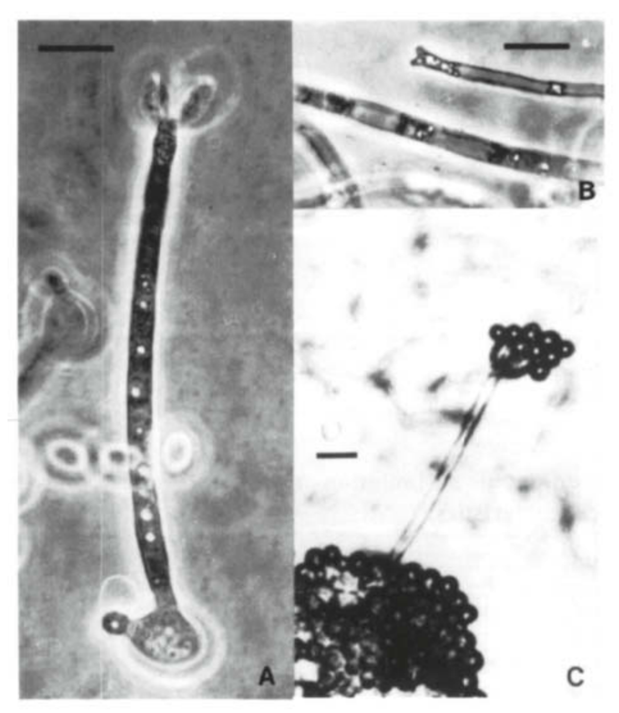


Fig. 366. *X. dendrorhous.* After 2 weeks at 20°C on Difco yeast nitrogen base agar with 0.5% glucitol. (A) Basidium with basidiospores formed following conjugation between a cell and its bud. (B) Basidium with terminal pegs that are attachment sites for basidiospores. (C) Attached basidiospores germinating by budding. Bars = 10 µm.

Life cycle: Formation of basidiospores is observed on agar media containing polyols (ribitol, D-glucitol, L-arabitol or D-xylitol), pentoses (D-ribose, D-xylose or D-arabinose) or D-lyxose, except for D-mannitol and L-arabinose, after 2–3 weeks at 18°C. Following conjugation between a cell and its bud, a slender cylindrical holobasidium forms which is 2 to 3.5 µm in diameter, and 30–165 µm (usually, 70–80 µm) in length (Fig. 366A). Rarely does conjugation occur between independent cells nor does a basidium form without apparent conjugation. Usually 3–4 (up to 6) thin-walled oval or ellipsoidal spores, 3.4–6.0×5.0–12.0 µm (av. 4×8 µm), are produced

[1] The information in this paragraph was added by the Editors to allow comparison with other genera.

[2] Golubev (1995) proposed, on the basis of descriptions by Ludwig (1891, 1896), that *Rhodomyces dendrorhous* represents the anamorph of *X. dendrorhous.* With the exception of drawings in the original publications, type material of *R. dendrorhous* is not available for comparison. Because the name *Phaffia rhodozyma* is of current economic importance, a proposal is being made to conserve this name against *R. dendrorhous* as the anamorphic name of *X. dendrorhous.* In view of this development, the editors have listed *P. rhodozyma* as the anamorph of *X. dendrorhous.*

terminally on the apex of the basidium giving it a flower-like appearance in apical view. Basidiospores are not forcibly discharged. They are formed on minute pegs (Fig. 366B) but appear sessile and germinate by budding (Fig. 366C).

Fermentation:

Glucose	s	Raffinose	w
Galactose	–	Trehalose	w
Sucrose	w	Cellobiose	w
Maltose	w	Melezitose	s
Lactose	–	Soluble starch	w/–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	w/–	Ethanol	s
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	w/–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	w/–
Raffinose	+	α-Methyl-D-glucoside	w/–
Melezitose	+	Salicin	s
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	s
D-Xylose	s	Succinate	s
L-Arabinose	+	Citrate	s
D-Arabinose	w/–	Inositol	–
D-Ribose	w/–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Thiamine-free	+
5-Keto-D-gluconate	w	Pyridoxine-free	+
D-Glucuronate	v	Xylan	–
Xylitol	w	Pectin	+
L-Arabinitol	w	Erythrose	–
Arbutin	+	β-Methyl-D-glucoside	+
Cadaverine	w	β-Methyl-D-arabinoside	–
Creatine	–	Aesculin	+
Creatinine	–	Ascorbate	–
L-Lysine	w	Mucate	–
Ethylamine	–	Fumarate	s
50% Glucose yeast extract agar	w	Oxalate	–
		Propionate	–
10% NaCl/5% glucose	–	Acetate	s
Starch formation (pH independent)	+	Allantoin	+
		Uric acid	s
Urease	+	Formamide	s
Nitrite	–	Acetamide	–
Gelatin liquefaction	w	Thioacetamide	–
Glucono-δ-lactone	s	Growth at 25°C	w/–
L-Tartaric acid	–	Growth at 30°C	–
L-Malic acid	ws		
Biotin-free	–		

Co-Q: 10 (Sugiyama et al. 1985).

Mol% G+C: 48.3 (BD: Miller et al. 1976b).

Extracellular polysaccharide hydrolyzates: Mannose, xylose, glucose, galactose and rhamnose.

Synthesis of carotenoid pigments: Positive, approximately 85% is astaxanthin (Andrewes et al. 1976).

Origin of the strains studied: Slime fluxes of deciduous trees: alder (*Alnus japonica*) (1), birch (*Betula maximowicziana*) (1), *B. tauschii* (1), dogwood (*Cornus brachypoda*) (2), beech (*Fagus crenata*) (1), all from Japan; *B. verrucosa* from Russia (10); *B. papyifera* (1) from Alaska; *Betula* sp. (1) from Finland.

Type strain: VKM Y-2786 (CBS 7918, JCM 9681), isolated from *Betula verrucosa* flux, Russia.

Comments on the genus

Xanthophyllomyces dendrorhous is an inhabitant of spring sapwood flows of deciduous trees and due to the abundant development of the yeast, the flowing sap assumes a salmon-red color (Golubev et al. 1977b). Ludwig (1891, 1896) reported a similar phenomenon caused by a yeast that he described as *Rhodomyces dendrorhous,* and which Golubev (1995) considered to be the anamorph of *X. dendrorhous.* Placement of *X. dendrorhous* among the basidiomycetous yeasts has been determined from comparisons using the strains designated as *Phaffia rhodozyma,* hence, the ensuing discussion will use this latter name. From the primary and secondary structure of 5S rRNA (Gottschalk and Blanz 1985), presence of xylose in the capsular polysaccharide (Sugiyama et al. 1985, Weijman and Rodrigues de Miranda 1988), sensitivity to mycocins produced by *Cystofilobasidium bisporidii* and *Cryptococcus laurentii* (Golubev and Kuznetsova 1989, Golubev 1990b), ability to synthesize starch-like compounds (pH independent) and assimilation of D-glucuronate, a tremellaceous (filobasidiaceous) affinity is suggested for *P. rhodozyma.* Based on these observations, the name *Phaffia* was regarded by Weijman et al. (1988) as a synonym of *Cryptococcus.* However, comparisons of partial 18S and 26S rRNA sequences showed that *Phaffia* does not cluster with *Cryptococcus,* but that it is close to *Cystofilobasidium* (Guého et al. 1989, Nakase et al. 1993, Yamada and Kawasaki 1989b, Yamada et al. 1990a) and *Mrakia* (Fell et al. 1995).

Golubev (1995) established that polyols and pentoses induce conjugation between a cell and its bud (pedogamy) in *X. dendrorhous,* and the subsequent formation, without production of mycelium, of a slender holobasidium that forms oval basidiospores at the apex. Cultivation on polyol-containing media appears to simulate the natural habitat when spring sapwood flow ceases and the flux appears dry. In response to water stress, eukaryotic microorganisms, which are inhabitants of the spring sap flows, accumulate high concentrations of polyhydric alcohols (Brown 1978), and this change in physiology appears to trigger sexual reproduction in *X. dendrorhous.*

The strains of this species that were studied are rather homogenous in their cultural, morphological, and physiological properties, and their isoenzyme and rDNA profiles (Varga et al. 1995). There are only minor differences in color intensity, cell sizes, starch fermentation and in the assimilation of those carbon compounds that are weakly utilized. However, significant differences in RAPD patterns and electrophoretic karyotypes were found among strains (Nagy et al. 1994, Adrio et al. 1995).

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Part VIc

Descriptions of anamorphic basidiomycetous genera and species

Contents

92. *Bensingtonia* Ingold emend. Nakase & Boekhout

T. Boekhout and T. Nakase

Diagnosis of the genus

Cells are ellipsoidal, ovoidal or elongate. Budding is mostly polar, with buds sessile or on short denticles, enteroblastic, and with percurrent or sympodial proliferation. Ballistoconidia are bilaterally symmetrical, straight or curved, apiculate, ellipsoidal, ovoidal, amygdaliform, falcate, allantoid or reniform. Colonies are whitish, cream, pale pink, brownish-yellow (ochre), brownish or grayish-red. True mycelia and/or pseudomycelia may be present. Septal pores are simple. Clamp connections are absent.

Fermentation is absent. D-Glucuronate and nitrate may be assimilated; *myo*-inositol is not assimilated. Diazonium blue B and urease reactions are positive. Xylose is absent from whole-cell hydrolyzates. The major ubiquinone is Q-9.

Type species

Bensingtonia ciliata Ingold

Species accepted

1. *Bensingtonia ciliata* Ingold (1986)
2. *Bensingtonia ingoldii* Nakase & M. Itoh (1989)
3. *Bensingtonia intermedia* (Nakase & M. Suzuki) Nakase & Boekhout (1988)
4. *Bensingtonia miscanthi* (Nakase & M. Suzuki) Nakase & Boekhout (1988)
5. *Bensingtonia naganoensis* (Nakase & M. Suzuki) Nakase & Boekhout (1988)
6. *Bensingtonia phyllada* (van der Walt & Y. Yamada) van der Walt, Nakagawa & Y. Yamada ex Boekhout (1991)
7. *Bensingtonia subrosea* (Nakase & M. Suzuki) Nakase & Boekhout (1988)
8. *Bensingtonia yamatoana* (Nakase, M. Suzuki & M. Itoh) Nakase & Boekhout (1988)
9. *Bensingtonia yuccicola* (Nakase & M. Suzuki) Nakase & Boekhout (1988)

Table 72
Key characters of species in the genus *Bensingtonia*

Species	Assimilation[a]																			
	Sor	Suc	Mal	Cel	Tre	Lac	Mel	Raf	Mlz	Sst	L-Ar	D-Ar	Rib	Rha	Eth	Ery	α-M	Sal	2Kg	Nit
Bensingtonia ciliata	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+
B. ingoldii	−	+	−	+	+	+	+	+	+	+	+	−	+	−	+	+	−	−	+	+
B. intermedia	+	v	v	+	+	−	−	−	v	−	−	v	+	−	+	−	−	−	+	−
B. miscanthi	+	+	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+
B. naganoensis	−	+	+	+	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	+
B. phyllada	+	+	+	+	+	+	−	−	+	+	+	−	−	+	+	−	+	+	−	+
B. subrosea	+	+	+	+	+	+	−	+	−	+	−	−	−	−	+	−	−	+	−	−
B. yamatoana	−	+	+	−	+	−	−	−	+	v	−	−	−	−	+	−	−	−	+	−
B. yuccicola	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+

[a] Abbreviations: Sor, L-sorbose; Suc, sucrose; Mal, maltose; Cel, cellobiose; Tre, trehalose; Lac, lactose; Mel, melibose; Raf, raffinose; Mlz, melezitose; Sst, soluble starch; L-Ar, L-arabinose; D-Ar, D-arabinose; Rib, D-ribose; Rha, L-rhamnose; Eth, ethanol; Ery, erythritol; α-M, α-methyl-D-glucoside; Sal, salicin; 2Kg, 2-keto-D-gluconate; Nit, nitrate.

Key to species

See Table 72.

1. a 2-Keto-D-gluconate assimilated → 2
   b 2-Keto-D-gluconate not assimilated → 4
2(1). a L-Sorbose assimilated .... *B. intermedia*: p. 725
   b L-Sorbose not assimilated → 3
3(2). a L-Arabinose, cellobiose, melibiose, erythritol and nitrate assimilated .... *B. ingoldii*: p. 724
   b L-Arabinose, cellobiose, melibiose, erythritol and nitrate not assimilated .... *B. yamatoana*: p. 729
4(1). a Nitrate assimilated → 5
   b Nitrate not assimilated .... *B. subrosea*: p. 728

5(4).	a Nitrite assimilated → 6		
	b Nitrite not assimilated	*B. ciliata*:	p. 724
6(5).	a Melibiose assimilated, trehalose not assimilated	*B. naganoensis*:	p. 726
	b Melibiose not assimilated, trehalose assimilated → 7		
7(6).	a Maltose and α-methyl-D-glucoside assimilated	*B. phyllada*:	p. 727
	b Maltose and α-methyl-D-glucoside not assimilated → 8		
8(7).	a Sucrose assimilated	*B. miscanthi*:	p. 726
	b Sucrose not assimilated	*B. yuccicola*:	p. 729

Systematic discussion of the species

92.1. *Bensingtonia ciliata* Ingold (1986)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ovoidal to ellipsoidal, (8.5–10.0)×(5.0–7.0) μm, and single. Budding is polar, with the buds sessile or on short denticles and with sympodial or percurrent proliferation (Fig. 367). Cells sometimes germinate with unbranched or irregularly branched hyphae, 2.0–3.5 μm wide, in which septa and retraction septa occur. Colonies are butyrous, smooth, glabrous or somewhat pruinose, shiny or dull, whitish to pale pinkish-cream, with the margin entire.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, no mycelium or pseudomycelium is formed. Aerobic growth is whitish-cream, shiny, butyrous, smooth, with the center slightly raised, and the margin entire.

Formation of ballistoconidia: Ballistoconidia are formed terminally at hyphae or hyphal branchlets on malt extract agar or on V8 juice agar. They are ovoidal or ellipsoidal to amygdaliform, (6.0–10.0)×(2.5–4.2) μm (Fig. 367).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	w	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	w
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	–
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 9, CBS 7514 (Boekhout 1987).
Mol% G+C: 45.0, CBS 7514 (T_m: Boekhout 1991a).

Origin of the strains studied: CBS 7514 (IMI 291091), *Auricularia auricula-judae* var. *lacteae* Quél., C.T. Ingold, Great Britain, and a morphological mutant derived from this strain (Ingold 1988).

Type strain: IMI 291091.

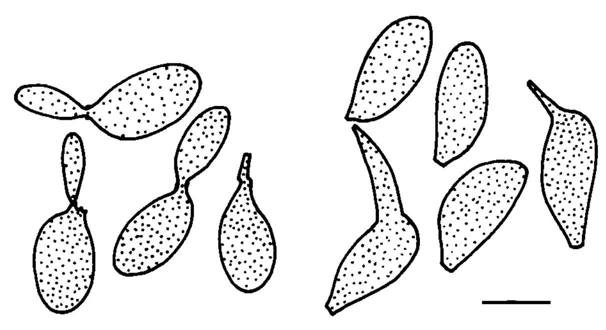


Fig. 367. *B. ciliata,* CBS 7514. Yeast cells and ballistoconidia on malt extract agar. Bar = 5 μm.

Comments: According to Ingold (1986) the ballistoconidia of this strain measure (12.0–15.0)×(5.0–6.0) μm on 0.2% malt extract agar. Based on our measurements ((6.0–10.0)×(2.5–4.2) μm) on 5% malt extract agar and V8 juice agar, one has to consider a large variability in the size of the ballistoconidia, depending on the media used. Contrary to the report of Nakase and Boekhout (1988), we found *B. ciliata* nitrate positive.

92.2. *Bensingtonia ingoldii* Nakase & M. Itoh (Nakase et al. 1989a)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ovoidal, ellipsoidal, fusoidal to subglobose, (6.0–10.0)×(3.5–6.0) μm, single or in short chains. Budding is polar, with the buds sessile or on short denticles and with sympodial proliferation (Fig. 368). Colonies are butyrous, smooth, dull, grayish-cream, with the margin entire.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate on morphology agar: After 7 days at 17°C, no mycelium or pseudomycelium is formed. Aerobic growth is yellowish-cream, dull to somewhat shiny, butyrous, smooth with the center somewhat raised, and the margin entire.

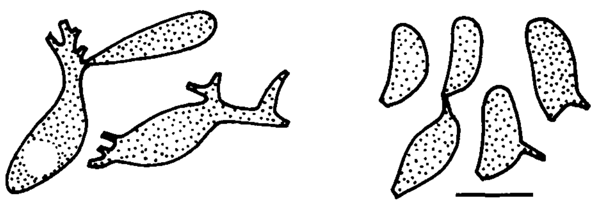


Fig. 368. *B. ingoldii*, CBS 7424. Yeast cells and ballistoconidia on malt extract agar. Bar = 5 µm.

Formation of ballistoconidia: Ballistoconidia are formed on sterigmata of up to ca. 10 µm long on malt extract, V8 juice, corn meal, and yeast–malt extract agars. They are reniform, and 6.0–9.0 × 2.0–4.2 µm (Fig. 368). Ballistoconidia sometimes form many sterigmata, giving the cells a star-like appearance.

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	l	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	s	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 9, JCM 7445 (Nakase et al. 1989a).
Mol% G + C: 55.7, JCM 7445 (HPLC: Nakase et al. 1989a).

Origin of the strain studied: CBS 7424 (JCM 7445), leaf of *Knightia excelsa*, J. Sugiyama, New Zealand.

Type strain: JCM 7445.

Comments: *Bensingtonia ingoldii* is physiologically similar to *B. intermedia*. *B. ingoldii* differs from the latter species by the presence of sympodially branched denticles or sterigmata, a somewhat lower mol% G + C content (Nakase et al. 1989a), and assimilation of L-arabinose, lactose, melibiose, *meso*-erythritol, and nitrate.

92.3. *Bensingtonia intermedia* (Nakase & M. Suzuki) Nakase & Boekhout (1988)

Synonyms:
Bullera intermedia Nakase & M. Suzuki (1986b)
Sporobolomyces intermedius (Nakase & M. Suzuki) Nakase & M. Suzuki (1987c)
Sporobolomyces weijmanii Nakase & M. Suzuki (1987c)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ovoidal, fusoidal or cylindrical, straight or slightly curved, (9–30) × (2.5–7.0) µm, and frequently in branched short chains. Budding is polar, with the buds showing sessile and sympodial proliferation. Colonies are butyrous, smooth, dull, cream, with the margin entire.

Growth on the surface of assimilation media (glucose): A ring, islets, and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, a pseudomycelium is formed (Fig. 369); cells may be septate. Aerobic growth is creamish-white, shiny, butyrous, flat, smooth or lightly reticulate, with the margin entire.

Formation of ballistoconidia: Ballistoconidia are formed on morphology, malt extract, corn meal, and potato dextrose agars. They are amygdaliform, ellipsoidal to reniform, and curved, (6.0–11.0) × (2.0–5.0) µm (Fig. 369).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	v	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

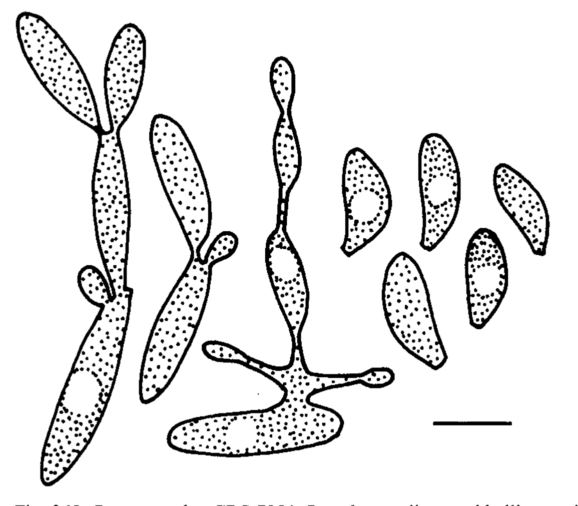


Fig. 369. *B. intermedia*, CBS 7281. Pseudomycelium and ballistoconidia on morphology agar. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	−
50% Glucose	−	Growth at 25°C	+
Starch formation	−	Growth at 30°C	−
Urease	+		

Co-Q: 9, JCM 5291, JCM 5651 (Nakase and Suzuki 1986b, 1987c)
Mol% G+C: 57.8–60.0, JCM 5291, JCM 5651 (T_m: Nakase and Suzuki 1986b, 1987c).

Origin of the strains studied: CBS 7226 (JCM 5291), dead leaf of rice *(Oryza sativa),* T. Nakase, Japan; CBS 7281 and 7241 (both JCM 5651), ex dead leaf *Miscanthus sinensis,* T. Nakase, Japan.
Type strain: JCM 5291.

Comments: Yamada and Nakagawa (1988) regarded *B. intermedia* and *Sporobolomyces weijmanii* conspecific based on the electrophoretic comparison of seven enzymes. However, several nutritional differences exist between the type strains of *B. intermedia* and *S. weijmanii,* e.g., assimilation of D-xylose, sucrose, maltose, melezitose and ribitol. The cell wall is layered and electron dense (Boekhout et al. 1992b).

92.4. *Bensingtonia miscanthi* (Nakase & M. Suzuki) Nakase & Boekhout (1988)

Synonym:
Sporobolomyces miscanthi Nakase & M. Suzuki (1987c)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ellipsoidal, fusoidal to cylindrical, straight or somewhat curved, (7.0–14.0)×(2.0–4.0) μm, and single. Budding is polar or lateral, with the buds sessile or on short denticles and with sympodial proliferation. Colonies are butyrous, smooth, shiny, pale grayish-cream, with the margin entire or somewhat eroded.

Growth on the surface of assimilation media (glucose): A ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, no pseudomycelium or mycelium is formed. Aerobic growth is pale pinkish-cream, dull, butyrous, with a slightly irregular surface, flat or slightly raised, and with the margin entire or somewhat crenulate.

Formation of ballistoconidia: Ballistoconidia are abundantly formed on potato dextrose, corn meal, malt extract, and morphology agars. They are reniform, allantoidal, or ellipsoidal. On corn meal agar they measure (9.0–17.0)×(4.3–5.5) μm, and on the lid of the petri dish (5.0–8.5)×(4.0–5.2) μm (Fig. 370).

Fermentation: absent.

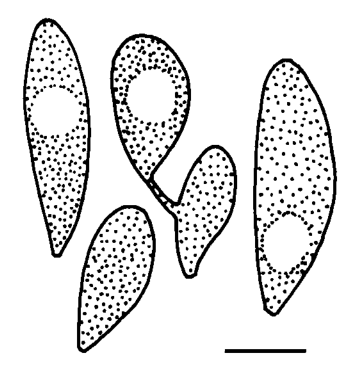


Fig. 370. *B. miscanthi,* CBS 7282. Ballistoconidia on corn meal agar. Bar = 5 μm.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	−	Methanol	−
L-Sorbose	l	Ethanol	−
Sucrose	+	Glycerol	+
Maltose	−	Erythritol	−
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	−
Lactose	l	D-Mannitol	+
Melibiose	−	D-Glucitol	+
Raffinose	l	α-Methyl-D-glucoside	−
Melezitose	−	Salicin	−
Inulin	−	D-Gluconate	−
Soluble starch	−	DL-Lactate	w
D-Xylose	l	Succinate	+
L-Arabinose	−	Citrate	+
D-Arabinose	−	Inositol	−
D-Ribose	−	Hexadecane	n
L-Rhamnose	−	Nitrate	+
D-Glucosamine	−	Vitamin-free	−

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	−	Nitrite	+
50% Glucose	−	Growth at 25°C	+
Starch formation	−	Growth at 30°C	−
Urease	+		

Co-Q: 9, JCM 5733, NB-151 (Nakase and Suzuki 1987c).
Mol% G+C: 47.2–47.5, two strains, JCM 5733, NB-151 (T_m: Nakase and Suzuki 1987c).

Origin of the strain studied: CBS 7282 (JCM 5733), dead leaves of *Miscanthus sinensis,* T. Nakase, Japan.
Type strain: JCM 5733.

Comments: Cell shape varies considerably depending on the substrate of growth. On potato dextrose agar and on morphology agar, *Sterigmatomyces*-like sterigmata are formed. Ballistoconidia frequently germinate with short acropetal chains of budding cells.

92.5. *Bensingtonia naganoensis* (Nakase & M. Suzuki) Nakase & Boekhout (1988)

Synonym:
Sporobolomyces naganoensis Nakase & M. Suzuki (1987a)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are fusoidal to ellipsoidal, (8.0–10.0)×(2.3–4.3) μm, and single. Budding is polar, rarely lateral, with the buds sessile or on short denticles and with

percurrent or sympodial proliferation. Colonies are rather dry, smooth, dull, pale grayish-cream, with the margin entire.

Growth on the surface of assimilation media (glucose): A ring and a sediment are formed.

Dalmau plate on morphology agar: No pseudomycelium or mycelium is formed. Aerobic growth is cream, dull, butyrous, nearly flat, with the surface weakly transversely ridged, and the margin entire.

Formation of ballistoconidia: Ballistoconidia are abundantly formed on morphology, corn meal and malt extract agars. They are amygdaliform, ellipsoidal to allantoid, (6.0–10.0)×(3.0–4.2) μm (Fig. 371).

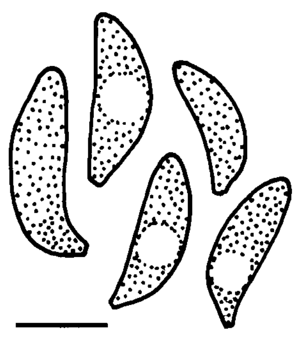


Fig. 371. *B. naganoensis,* CBS 7286. Ballistoconidia on malt extract agar. Bar = 5 μm.

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	l
Soluble starch	+	DL-Lactate	l
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 9, JCM 5978 (Nakase and Suzuki 1987a).

Mol% G + C: 55.8, JCM 5978 (T_m: Nakase and Suzuki 1987a).

Origin of the strain studied: CBS 7286 (JCM 5978), dead leaf of *Sasa* sp., T. Nakase, Japan.

Type strain: JCM 5978.

Comments: *Bensingtonia naganoensis* can be differentiated from both *B. miscanthi* and *B. subrosea,* by its inability to assimilate trehalose, lactose and its ability to grow on melibiose and melezitose. On malt extract agar, long lysed sterigmata are sometimes present.

92.6. *Bensingtonia phyllada* (van der Walt & Y. Yamada) van der Walt, Nakagawa & Y. Yamada ex Boekhout (1991a)

Synonyms:

Sporobolomyces phylladus (as *S. phyllades*) van der Walt & Y. Yamada (van der Walt et al. 1989a)

Bensingtonia phylladus (van der Walt & Y. Yamada) Y. Yamada, Nakagawa & van der Walt (1988c) nom. inval.

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ellipsoidal, broadly ellipsoidal, ovoidal or subglobose, (8.0–13.0)×(5.0–8.0) μm, and single. Budding is polar or lateral, with the buds sessile or on short denticles. Cells may develop 1.5–3.5 μm wide branched hyphae, in which retraction septa occur, and with the cytoplasm retracted in certain cells. Colonies are butyrous, smooth, dull or somewhat shiny, whitish-cream, and with the margin somewhat eroded because of submerged marginal hyphal growth.

Growth on the surface of assimilation media (glucose): A ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, short hyphae with retraction septa may develop. Aerobic growth is whitish to pinkish-cream, somewhat shiny or dull, butyrous, nearly flat, smooth or somewhat transversely reticulate, with the margin entire or somewhat crenulate.

Formation of ballistoconidia: Ballistoconidia are formed on morphology, malt extract and corn meal agars. They are ellipsoidal, amygdaliform, allantoid to falcate, (9.0–14.0)×(4.0–7.0) μm (on the surface of malt extract agar ballistoconidia measure (14.0–21.0)×(5.0–3–7.0) μm) (Fig. 372).

Fermentation: absent.

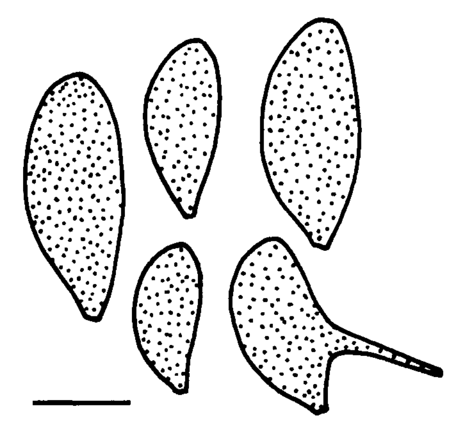


Fig. 372. *B. phyllada,* CBS 7169. Ballistoconidia on malt extract agar. Bar = 5 μm.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	w	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	w	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	w	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 9, CBS 7169 (van der Walt et al. 1989a).
Mol% G+C: 50.4, CBS 7169 (T_m: van der Walt et al. 1989a).

Origin of the strain studies: CBS 7169, leaves of *Sclerocarya caffra*, J.P. van der Walt, South Africa.
Type strain: CBS 7169.

Comments: The ultrastructure of the cell walls is electron dense and layered, and septa are simple diaphragma-like and attenuating towards the pore (Boekhout et al. 1992b). The observed pore structure suggests a close phylogenetic relationship with the genera *Sporobolomyces* and *Sporidiobolus*. Budding of yeast cells occurs percurrently (Boekhout, unpublished results).

92.7. *Bensingtonia subrosea* (Nakase & M. Suzuki) Nakase & Boekhout (1988)

Synonym:
Sporobolomyces subroseus Nakase and M. Suzuki (1987c)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ellipsoidal to cylindrical, (7.0–14.0)×(4.0–7.0) μm, and single. Budding is mostly polar, and occasionally lateral, with the buds sessile or on short denticles and with percurrent proliferation. Colonies are butyrous, smooth, shiny, grayish-cream, with the margin entire.

Growth on the surface of assimilation media (glucose): Absent, but a sediment may be formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, no pseudomycelium or mycelium is formed. Aerobic growth is pale pinkish-cream, shiny, butyrous, smooth, flat or slightly raised, and with the margin entire.

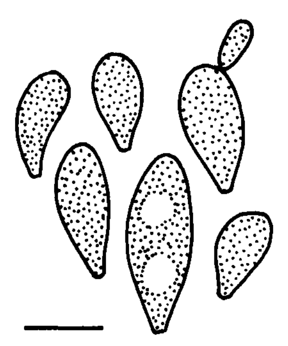


Fig. 373. *B. subrosea*, CBS 7283. Ballistoconidia on corn meal agar. Bar = 5 μm.

Formation of ballistoconidia: Ballistoconidia were observed on corn meal agar. They are ellipsoidal, and sometimes somewhat pointed toward the apex, (7.0–13.0)×(3.8–5.0) μm (Fig. 373).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	l	Ethanol	l
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	l
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	l	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	–
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 9, JCM 5735 (Nakase and Suzuki 1987c).
Mol% G+C: 46.5, JCM 5735 (T_m: Nakase and Suzuki 1987c).

Origin of the strain studied: CBS 7283 (JCM 5735), dead leaf of *Miscanthus sinensis,* T. Nakase, Japan.
Type strain: JCM 5735.

Comments: *Bensingtonia subrosea* is similar to *B. miscanthi* in morphology, nutritional physiology and mol% G+C. Due to a low similarity value of about 19% in the electrophoretic patterns of 10 enzymes in both species, Nakase and Suzuki (1987c) described them as separate species. Physiologically, *B. subrosea* can be differentiated from *B. miscanthi* by assimilation of maltose, soluble starch and the inability to assimilate nitrate and nitrite.

92.8. *Bensingtonia yamatoana* (Nakase, M. Suzuki & M. Itoh) Nakase & Boekhout (1988)

Synonym:
Sporobolomyces yamatoanus Nakase, M. Suzuki & M. Itoh (1987b)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are cylindrical, ellipsoidal or subglobose, straight or somewhat curved, (9.0–15.0)×(2.0–4.0) µm. Septate hyphae are present, in which retraction septa are formed. Conidiogenesis is polar, with the buds sessile or on terminal sterigma-like outgrowths and with percurrent proliferation. Blastoconidia are formed on hyphae. Colonies are rather tough or butyrous, dry, flat or with the center raised, smooth or somewhat irregular, dull, pale yellowish-brown, and the margin may be entire or eroded.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, a septate mycelium is formed. Cylindrical, oblong or ellipsoidal blastoconidia are formed laterally and terminally on hyphae or sterigma-like outgrowths. Aerobic growth is variable, yellowish-cream, yellowish, or yellowish-brown, dull, tough, dry, or butyrous, with the surface nearly smooth, or irregular, low pustulate, transversely wrinkled to reticulate, with the margin crenulate, entire or eroded.

Formation of ballistoconidia: Ballistoconidia are formed laterally or terminally on hyphae on corn meal agar. They are reniform, falcate or allantoid, (9.0–20.0)×(3.0–6.0) µm (Fig. 374).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	w
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	v
Soluble starch	v	DL-Lactate	–
D-Xylose	l	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 9 (Nakase et al. 1987b).

Mol% G+C: 53.3–54.1, 5 strains, JCM 2896, JCM 5652, JCM 5975, JCM 6857, JCM 6858 (T_m: Nakase et al. 1987b).

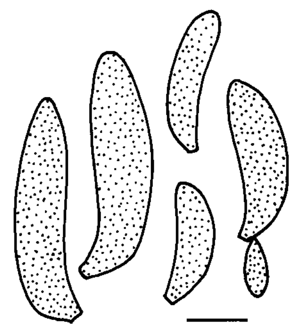


Fig. 374. *B. yamatoana,* CBS 7244. Ballistoconidia on corn meal agar. Bar = 5 µm.

Origin of the strains studied: CBS 7242 (JCM 5977), dead leaf of *Sasa* sp., T. Nakase, Japan; CBS 7243 (JCM 2896), CBS 7244 (JCM 5652), both from dead leaf of *Miscanthus sinensis,* T. Nakase, Japan; CBS 7245 (JCM 5975), dead leaf of *Sasa* sp., T. Nakase, Japan.

Type strain: JCM 2896 (CBS 7243).

Comments: *Bensingtonia yamatoana* can be readily distinguished from all other *Bensingtonia* species because of the following characteristics: assimilation of 2-keto-D-gluconate, abundant formation of hyphae and rather dark yellow (ochre) colonies, and large ballistoconidia. Under TEM, septa of CBS 7243 have a central narrow pore. The cell walls are electron dense and layered (Boekhout et al. 1992b).

92.9. *Bensingtonia yuccicola* (Nakase & M. Suzuki) Nakase & Boekhout (1988)

Synonym:
Sporobolomyces yuccicola Nakase & M. Suzuki (1988)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ellipsoidal, (7.0–11.0)×(3.5–6.5) µm, and single. Budding is polar or nearly polar, with the buds sessile or on short denticles and with percurrent proliferation. Colonies are butyrous, smooth, shiny, grayish-cream, with the margin entire.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate on morphology agar: After 7 days at 17°C, yeast cells can have short hyphal outgrowths, but there is no formation of extensive pseudohyphae or true hyphae. Aerobic growth is pale yellowish-cream, dull or shiny, butyrous, flat, with a smooth surface, flat, and with the margin entire, straight or somewhat crenulate.

Formation of ballistoconidia: No ballistoconidia were seen in strain CBS 7331. According to Nakase and Suzuki (1988) ballistoconidia, formed on corn meal agar, are apiculate or short ellipsoidal (broadly ellipsoidal) or ellipsoidal, (7.0–11.0)×(2.5–6.0) µm.

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 9, CBS 7331 (Nakase and Suzuki 1988).
Mol% G + C: 45.8–45.9, JCM 6251 (T_m: Nakase and Suzuki 1988).

Origin of the strain studied: CBS 7331 (JCM 6251), leaf of *Yucca* species, R.J. Bandoni, Canada.

Type strain: JCM 6251.

Comments on the genus

Ingold (1986) described *B. ciliata* as the only species of the genus *Bensingtonia.* Because whole-cell hydrolyzates lacked xylose and the coenzyme Q system comprised 9 isoprenologs (Boekhout 1987, 1991a), Nakase and Boekhout (1988) recombined several *Sporobolomyces* species of the *'intermedius'* group into *Bensingtonia.* Conidiogenesis of the genus in its current circumscription is variable. Most species show polar conidiogenesis with percurrent or sympodial proliferation. Some species, e.g., *B. ingoldii, B. miscanthi* and *B. subrosea,* form elongated denticles similar to that described in *Ballistosporomyces* (Nakase et al. 1989b). Morphologically, species of *Bensingtonia* cannot be differentiated from many of the recently described unpigmented species of *Sporobolomyces* (see key to species with bilaterally symmetrical ballistoconidia, ch. 76).

93. *Bullera* Derx

T. Boekhout and T. Nakase

Diagnosis of the genus

Cells are ellipsoidal, subglobose, or cylindroidal. Budding is mostly polar, and the buds are sessile or on short denticles, enteroblastic, and with percurrent or sympodial proliferation. Ballistoconidia are either rotationally or bilaterally symmetrical. If rotationally symmetrical, ballistoconidia are globose to subglobose, ellipsoidal, sometimes somewhat angular, transversely reniform, flabelliform, or turbinate, and if bilaterally symmetrical, ballistospores are reniform, allantoid or lunate. Colonies are whitish, yellowish, brownish, orange or reddish, smooth, reticulate or venose, mucoid, butyrous or rather dry. Hyphae or pseudohyphae may be present. Clamp connections are absent, but present in the dikaryophase.

Fermentation is absent. Diazonium blue B and urease reactions are positive. Xylose is present in whole-cell hydrolyzates. The major ubiquinone is Q-10.

Type species

Bullera alba (Hanna) Derx

Species accepted

1. *Bullera alba* (Hanna) Derx (1948): see *Bulleromyces albus,* p. 641
2. *Bullera armeniaca* Buhagiar (1983)
3. *Bullera crocea* Buhagiar (1983)
4. *Bullera dendrophila* van der Walt & D.B. Scott (1970)
5. *Bullera globispora* Johri & Bandoni (1984)
6. *Bullera megalospora* Nakase & M. Suzuki (1986)
7. *Bullera miyagiana* Nakase, M. Itoh, Takematsu & Bandoni (1990)
8. *Bullera oryzae* Nakase & M. Suzuki (1985)
9. *Bullera pyricola* Stadelmann (1975)
10. *Bullera pseudoalba* Nakase & M. Suzuki (1986)
11. *Bullera punicea* (Komagata & Nakase) Nakase & M. Suzuki (1986)
12. *Bullera sinensis* Li (1982)
13. *Bullera variabilis* Nakase & M. Suzuki (1987)

Key to species

See Table 73.

1.	a	Nitrate assimilated, lactate not assimilated; ballistoconidia bilaterally symmetrical → 2		
	b	Nitrate not assimilated, lactate usually assimilated; ballistoconidia more or less rotationally symmetrical → 4		
2(1).	a	Lactose and melibiose assimilated	*B. pyricola*:	p. 737
	b	Lactose and melibiose not assimilated → 3		
3(2).	a	D-Xylose assimilated	*B. megalospora*:	p. 735
	b	D-Xylose not assimilated	*B. punicea*:	p. 739
4(1).	a	α-Methyl-D-glucoside assimilated → 5		
	b	α-Methyl-D-glucoside not assimilated → 15		
5(4).	a	Nitrite assimilated → 6		
	b	Nitrite not assimilated → 8		
6(5).	a	Lactose, erythritol and inositol assimilated → 7		
	b	Lactose, erythritol and inositol not assimilated	*B. crocea*:	p. 733
7(6).	a	D-Glucosamine and galactitol assimilated	*B. oryzae*:	p. 736
	b	D-Glucosamine and galactitol not assimilated	*B. miyagiana*:	p. 736
8(5).	a	L-Sorbose assimilated → 9		
	b	L-Sorbose not assimilated → 12		
9(8).	a	Lactose assimilated → 10		
	b	Lactose not assimilated → 11		
10(9).	a	Inulin and glycerol assimilated	*B. alba*:	p. 732
	b	Inulin and, usually, glycerol not assimilated	*B. variabilis*:	p. 740
11(9).	a	Ethanol assimilated	*B. sinensis*:	p. 739
	b	Ethanol not assimilated	*B. variabilis*:	p. 740
12(8).	a	Raffinose assimilated → 13		
	b	Raffinose not assimilated	*B. dendrophila*:	p. 733

13(12). a Inulin assimilated .... *B. alba*: p. 732
b Inulin not assimilated → 14
14(13). a Ethanol assimilated .... *B. pseudoalba*: p. 738
b Ethanol not assimilated .... *B. variabilis*: p. 740
15(4). a Melezitose and D-glucosamine assimilated .... *B. armeniaca*: p. 732
b Melezitose and D-glucosamine not assimilated .... *B. globispora*: p. 734

Table 73
Key characters of species in the genera *Bulleromyces* and *Bullera*

Species	Assimilation[a]															Appearance[b]			Growth, 25°C	Mol% G+C
	Gal	Lac	Mel	Raf	Mlz	Inu	Xyl	Rha	Glu	Ery	Met	Lat	Cit	Ino	Nit	Ball.	Or.	Pi.		
Bulleromyces albus	+	+	v	+	+	+	+	+	v	v	+	+	+	+	–	R	–	–	+	53.5–54.5
Bullera armeniaca	+	–	–	+	+	–	+	+	+	–	–	+	–	+	–	R	+	–	+	54–57
B. crocea	+	–	+	+	+	–	+	+	–	–	+	+	+	–	–	R	+	–	+	55–57
B. dendrophila	+	+	–	–	+	–	+	+	–	–	+	+	+	+	–	R	–	–	+	39–40.5
B. globispora	+	–	–	+	–	–	+	+	–	–	–	+	+	+	–	R	–	–	+	53
B. megalospora	+	–	–	+	+	–	+	–	–	–	–	–	+	–	+	B	–	v	+	49–51
B. miyagiana	+	+	+	+	+	–	+	+	–	+	+	+	+	+	–	R	–	–	+	59–62
B. oryzae	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	R	–	–	+	63
B. pyricola	+	+	+	+	+	–	+	+	–	–	–	–	+	+	+	B	–	v	+	50–53.5
B. pseudoalba	+	+	+	+	+	–	+	+	+	–	+	+	+	+	–	R	–	v	+	52.2-54
B. punicea	–	–	–	+	+	–	–	v	–	–	–	–	v	v	+	B	–	v	–	54–54.5
B. sinensis	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	R	–	v	+	54.5–57
B. variabilis	+	+	+	+	+	–	+	+	+	v	+	+	+	+	–	R	–	–	+	56–57

[a] Abbreviations: Gal, galactose; Lac, lactose; Mel, melibose; Raf, raffinose; Mlz, melezitose; Inu, inulin; Xyl, D-xylose; Rha, L-rhamnose; -Glu, D-glucosamine; Ery, erythritol; Met, α-methyl-D-glucoside; Lat, DL-lactate; Cit, citrate; Ino, inositol; Nit, nitrate.
[b] Abbreviations: Ball., ballistospores, rotationally symmetrical (R) or bilaterally symmetrical (B); Or., orange color of colony; Pi., pink color of colony.

Systematic discussion of the species

93.1. *Bullera alba* (Hanna) Derx (1948)

See *Bulleromyces albus*, p. 641.

93.2. *Bullera armeniaca* Buhagiar (Buhagiar et al. 1983)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal, (5.0–10.0)×(2.0–4.0) µm, and single. Budding is polar and buds are sessile or on short denticles with percurrent or sympodial proliferation. Growth is butyrous, flat, with the center somewhat raised, shiny, pinkish-orange or orange, and with the margin entire or slightly eroded.

Growth on the surface of assimilation media (glucose): A thin film and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, a pseudomycelium occurs with cells measuring (10–110)×(3.0–4.5) µm. Aerobic growth is orange to brownish-orange, butyrous, flat, with the center somewhat raised, and with the margin entire, straight or somewhat crenulate.

Formation of ballistoconidia: Ballistoconidia, formed on corn meal, yeast–malt extract and malt extract agars, are subglobose to turbinate and measure (4.0–7.5)×(4.0–8.0) µm (Fig. 375).

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	w
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	+
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase 1987).

Mol% G+C: 54.3–56.6, two strains, CBS 7018, 7091 (T_m: Boekhout 1991a), 55.5 (HPLC: Nakase, unpublished results).

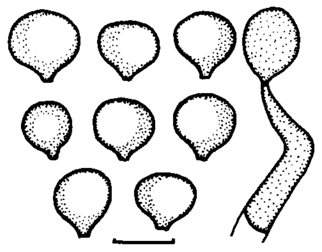


Fig. 375. *B. armeniaca*, CBS 7018. Ballistoconidia on corn meal agar. Bar = 5 μm.

Origin of the strains studied: CBS 7018, CBS 7091, cabbage, R.W.M. Buhagiar, Great Britain; CBS 7091.1, pale mutant of CBS 7091.

Type strain: CBS 7091.

Comments: Besides the typical orange colored strains, pale mutants do occur. From morphological and physiological points of view, *B. armeniaca* comes close to *B. crocea* Buhagiar (Buhagiar et al. 1983). *Bullera armeniaca* lacks the ability to assimilate melibiose and α-methyl-D-glucoside, and salicin is only weakly assimilated. *Bullera crocea* does not assimilate inositol at all, whereas assimilation of this compound is delayed by *B. armeniaca*. The reported difference in mol% G+C of 2.5 could not be confirmed by the present author using the T_m-method. However, when analyzed by HPLC, *B. crocea* has a lower mol% G+C of 51.6. These two taxa are also kept separate, because of low similarity of electrophoretic enzyme patterns (T. Nakase, personal communication).

93.3. *Bullera crocea* Buhagiar (Buhagiar et al. 1983)

Synonym:
Bullera aurantiaca Johri & Bandoni (1984)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal to subglobose, (6.0–11.0)×(4.0–7.0) μm, and single. Short hyphae may occur. Budding is polar and buds are sessile or on short denticles with percurrent or sympodial proliferation. Scars may be visible. Growth is soft, butyrous to rather dry, with the center slightly raised, smooth or reticulately ridged, shiny or dull, salmon-orange, orange, and with the margin entire, straight or strongly lobate.

Growth on the surface of assimilation media: A thin film and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, well-developed hyphae may be formed, with cells measuring (15–100)×(3.0–4.5) μm. Aerobic growth is orange to brownish-orange, soft to dry, flat, warty or ridged, with margin entire, straight or lobate.

Formation of ballistoconidia: Ballistoconidia, formed on morphology and corn meal agars, are subglobose to turbinate, and measure (4.0–8.0)×(4.0–9.0) μm (Fig. 376).

Fermentation: absent.

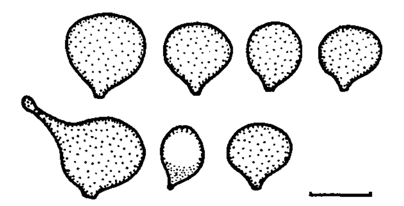


Fig. 376. *B. crocea*, CBS 6714. Ballistoconidia on corn meal agar. Bar = 5 μm.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	w	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase 1987).

Mol% G+C: 54.9–56.6, two strains, CBS 6714, 6980 (T_m: Boekhout 1991a), 51.6 (HPLC: Nakase, unpublished results).

Origin of the strains studied: CBS 6714, strawberries, R.W.M. Buhagiar, Great Britain; CBS 6714.1, pale mutant of CBS 6714; CBS 6980, type of *B. aurantiaca* Johri & Bandoni, stems of *Urtica*, R.J. Bandoni, Canada.

Type strain: CBS 6714.

Comments: Pale mutants occur besides the orange colored strains. CBS 6980 extensively forms hyphae on corn meal agar, on which short chains of blastoconidia are formed apically and laterally. Under TEM, cell walls of CBS 6714 are layered and electron dense. No pore structure has been observed (Boekhout, unpublished results).

93.4. *Bullera dendrophila* van der Walt & D.B. Scott (1970)

Synonym:
Aessosporon dendrophilum van der Walt (1973)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal to somewhat cylindroidal, (7.0–13.0)×(4.5–11.5) μm, and single. Budding is polar and buds are sessile or on short denticles. Growth is mucoid, with the center somewhat raised, smooth, glossy, cream to pale grayish-brown, and with the margin entire.

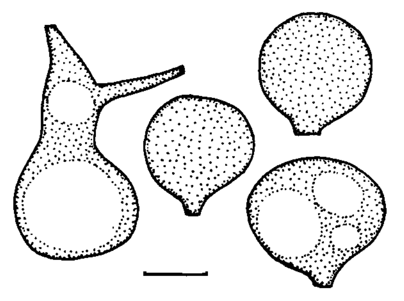


Fig. 377. *B. dendrophila*, CBS 6460. Ballistoconidia on corn meal agar. Bar = 5 µm.

Growth on the surface of assimilation media (glucose): A thin film, ring, and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, no mycelium or pseudomycelium is formed. Cells are ellipsoidal, cylindroidal to somewhat dumb-bell-shaped, or ovoidal. Aerobic growth is cream, butyrous, shiny, flat or somewhat raised, smooth or with faint transverse striations, with the margin entire, straight or somewhat crenulate.

Formation of ballistoconidia: Ballistoconidium formation is poor. Best results were obtained on corn meal agar at 17°C. Ballistoconidia are ellipsoidal to subglobose, and measure (4.5–9.0)×(5.5–10.5) µm (van der Walt and Scott 1970). On the surface of the agar plate, ballistoconidia measure (8.5–11.0)×(9.5–13.0) µm (Fig. 377).

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	s
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase 1987).

Mol% G+C: 38.8–40.4, two strains, CBS 6459, CBS 6074 (T_m: Boekhout 1991a), 38.4 (HPLC: Nakase, unpublished results).

Origin of the strains studied: CBS 6074, frass of larvae of *Buprestidae* in subcortical galleries of *Dichrostachys cinerea*, J.P. van der Walt, South Africa; CBS 6459 and CBS 6460, as *Aessosporon dendrophilum,* supposed diplophase of *B. dendrophila*, J.P. van der Walt, South Africa.

Type strain: CBS 6074.

Comments: Selected isolates of CBS 6074 have been interpreted as the perfect state of *Bullera dendrophila,* viz. *Aessosporon dendrophilum* van der Walt (van der Walt 1973), due to the presence of germinating chlamydospores on corn meal agar or potato glucose agar. However, it has not been proven that alternation of stages in the life cycle occurs in these cells, nor that ploidy differences are present. Therefore, we regard *A. dendrophilum* synonymous with *B. dendrophila.*

93.5. *Bullera globispora* Johri & Bandoni (1984)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ovoidal, ellipsoidal, cylindroidal or subglobose, (7.0–14.0)×(3.0–8.0) µm, and single. Septate hyphae are sometimes present. Budding is polar, with sympodial or percurrent proliferation, and collarette-like scars are visible. Growth is mucoid, flat or somewhat raised, smooth, glossy, pale grayish-brown, and with the margin entire.

Growth on the surface of assimilation media (glucose): A ring, a thin film and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, poorly developed pseudohyphae are formed. Cells are ellipsoidal or cylindroidal, and may be septate. Aerobic growth is pale yellowish-brown, butyrous to mucoid, shiny, flat or somewhat raised, with the surface smooth, or locally somewhat venose or reticulate. The margin is entire, straight or somewhat lobate.

Formation of ballistoconidia: Ballistoconidia, formed on morphology, malt extract, potato extract and corn meal agars, are subglobose, turbinate or transversely reniform, and measure (4.0–10.0)×(6.0–13.5) µm (Fig. 378).

Fermentation: absent.

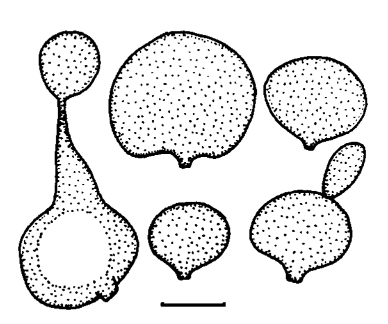


Fig. 378. *B. globispora*, CBS 6981. Ballistoconidia on morphology agar. Bar = 5 µm.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	v
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase 1987).

Mol% G+C: 52.9, one strain, CBS 6981 (T_m: Boekhout 1991a), 49.6 (HPLC: Nakase, unpublished results).

Origin of the strains studied: CBS 6981 (UBC 8079), UBC 70-8084, dead wood, B.N. Johri and R.J. Bandoni, Canada.

Type strain: UBC 8079 (CBS 6981).

Comments: According to Johri and Bandoni (1984), *B. globispora* has subglobose ballistoconidia. This feature was considered to be an important characteristic by these authors. However, in the two strains studied here, the ballistoconidia were generally broader than long. In this aspect they agree with the ballistoconidia of *B. armeniaca, B. pseudoalba* and *B. sinensis.* Moreover, the ballistoconidia we observed were considerably larger (viz. 4.0–10.0×6.0–12.5 µm) if compared with the data presented by Johri and Bandoni (l.c., 3.5–6.0×3.5–6.5 µm). Several differences were observed in the carbon assimilation pattern if compared with the data of Johri and Bandoni (l.c.), viz. no assimilation of L-sorbose, α-methyl-D-glucoside, melezitose, inulin, erythritol and assimilation of citrate. Physiologically, *B. globispora* is close to *B. armeniaca,* but it differs from that species by its inability to assimilate D-glucosamine and melezitose.

93.6. *Bullera megalospora* Nakase & M. Suzuki (1986c)

Synonym:

Udeniomyces megalosporus (Nakase & M. Suzuki) Nakase & Takematsu (1992)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal, (12–26)×(4.0–8.5) µm, and single. Budding is polar and buds are sessile, with percurrent proliferation and visible collarettes. Growth is butyrous, flat and somewhat raised in the center, smooth, shiny, whitish-cream, and with the margin entire, straight or somewhat undulate.

Growth on the surface of assimilation media (glucose): A thin film, islets, and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, part of the cells form short hyphal outgrowths, which may be branched. Cells are ellipsoidal, and somewhat fusiform or limoniform. Aerobic growth is cream or pale pinkish-cream, shiny, flat or with the center somewhat raised, with the margin entire or slightly eroded, and straight or somewhat crenulate.

Formation of ballistoconidia: Ballistoconidia, formed on morphology, malt extract, yeast–malt extract and corn meal agars, are bilaterally symmetrical, lunate, allantoid or ellipsoidal, and measure (11.0–18.0)×(5.0–14.0) µm (Fig. 379).

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	w	Methanol	–
L-Sorbose	w	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	l	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	v	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1986c).

Mol% G+C: 48.8–50.9, three strains, JCM 5269, NO-59, NO-102 (T_m: Nakase and Suzuki 1986c).

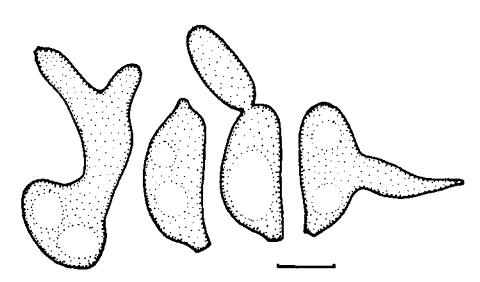


Fig. 379. *B. megalospora,* CBS 7236. Ballistoconidia on corn meal agar. Bar = 5 µm.

Origin of the strains studied: CBS 7236 (JCM 5269), CBS 7339 (JCM 5272), CBS 7355 (JCM 5271), CBS 7356 (JCM 5273), all four from dead leaves of rice (*Oryza sativa*), T. Nakase, Japan; CBS 7340 (JCM 5711), CBS 7359 (JCM 5712), both from dead leaves of *Miscanthus sinensis*, T. Nakase, Japan; CBS 7363 (JCM 5870), *Sasa* species, T. Nakase, Japan.

Type strain: JCM 5269 (CBS 7236).

Comments: *Bullera megalospora* can physiologically be differentiated from *B. punicea* by its inability to assimilate inositol. The other *Bullera* species with bilaterally symmetrical ballistospores, *B. pyricola*, differs by the ability to assimilate melibiose, lactose, galactitol and inositol.

93.7. *Bullera miyagiana* Nakase, M. Itoh, Takematsu & Bandoni (1990a)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal, limoniform to subglobose, (7.0–10.0)×(5.5–6.0) µm, and single. Budding is polar, on denticles or short stalks, with percurrent proliferation. Short hyphae may be present, up to ca. 180 µm long and 3.0–4.0 µm wide. Growth is butyrous, flat to slightly raised, somewhat venose, dull, pale yellowish, and with the margin entire, straight or slightly crenulate.

Growth on the surface of assimilation media: A ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, neither hyphae nor pseudohyphae are formed. Cells are ellipsoidal to subglobose. Aerobic growth is pale yellowish, dull, flat to slightly raised, with the margin entire, straight or somewhat crenulate. Hyphae are formed on malt extract agar and corn meal agar.

Formation of ballistoconidia: Ballistoconidia, formed on malt extract agar, are rotationally symmetrical, subglobose to flabelliform, and measure (5.5–10.5)×(5.0–11.0) µm (Fig. 380).

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	w	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

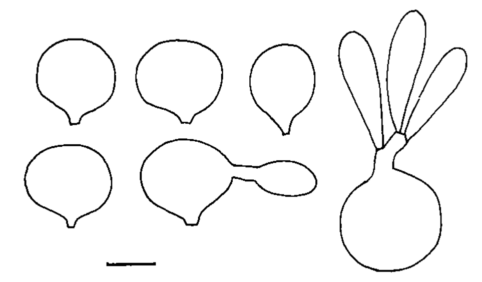


Fig. 380. *B. miyagiana*, CBS 7526. Ballistoconidia on malt extract agar. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	+
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase et al. 1990a).

Mol% G+C: 61.4, JCM 7536 (T_m: Nakase et al. 1990a), 58.9, JCM 7536 (HPLC: Nakase et al. 1990a).

Origin of the strain studied: CBS 7526 (JCM 7536), *Abies firma*, R.J. Bandoni, Japan.

Type strain: JCM 7536.

Comments: *Bullera miyagiana* is close to *B. oryzae*, and differs by growth with ethanol, and lack of growth with D-glucosamine, galactitol and nitrate. Ballistoconidia of *B. miyagiana* germinate by sympodial proliferation (Fig. 380) (Boekhout 1991a).

93.8. *Bullera oryzae* Nakase & M. Suzuki (1985c)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal to subglobose, (5.0–10.0)×(3.0–6.0) µm, and single. Budding is polar and buds are sessile or on short denticles. Growth is butyrous, flat, smooth, shiny, pale grayish-brown, and with entire margins.

Growth on the surface of assimilation media (glucose): A ring, islets, a film, and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, no pseudohyphae or true hyphae are formed, but cells may form short hyphal-like outgrowths. Cells are ellipsoidal to subglobose and frequently somewhat thick-walled with the outer cell wall layer peeling off. Aerobic growth is pale yellow or brownish-yellow, with the margin slightly discolorous, dull, somewhat raised, flat or weak venose. The margin is entire, straight, lobate or crenulate.

Formation of ballistoconidia: Ballistoconidia, formed on corn meal agar, are globose to subglobose, and measure (4.5–8.0)×(4.0–6.5) µm (Fig. 381). Occasionally, huge globose to subglobose ballistoconidia were observed on corn meal agar, measuring (10.0–13.0)×(8.0–10.0) µm (Fig. 381).

Fermentation: absent.

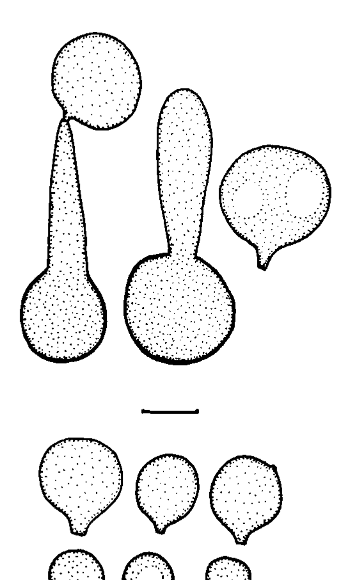


Fig. 381. *B. oryzae,* CBS 7194 (top) and CBS 7342 (bottom). Ballistoconidia on corn meal agar. Bar = 5 µm.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	l
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	+
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase and Suzuki 1985c).

Mol% G + C: 62.7, JCM 5281 (T_m: Nakase and Suzuki 1985c), 59.7 (HPLC: Nakase, unpublished results).

Origin of the strains studied: CBS 7194 (JCM 5281), dead leaf of rice (*Oryza sativa*), T. Nakase, Japan; CBS 7341 (JCM 5718), CBS 7360 (JCM 5719), CBS 7361 (JCM 5728), all three from dead leaves of *Miscanthus sinensis*, T. Nakase, Japan; CBS 7342 (JCM 5987), leaf of *Sasa* species, T. Nakase, Japan.

Type strain: JCM 5281 (CBS 7194).

Comments: *Bullera oryzae* belongs to the group of species similar to *B. alba,* and can be readily identified because of assimilation of nitrite. *Bullera oryzae* differs from both *B. sinensis* and *B. pseudoalba* by forming ballistoconidia that are more globose to subglobose. Moreover, *B. sinensis* can be separated by the assimilation of L-sorbose and its inability to use lactose. *Bullera pseudoalba* differs by growth on glycerol (Boekhout 1991a) and its inability to assimilate erythritol. Mol% G + C of *Bullera alba* differs by 8.3% from *B. oryzae.*

93.9. *Bullera pyricola* Stadelmann (1975)

Synonyms:

Udeniomyces pyricola (as *U. piricola*) (Stadelmann) Nakase & Takematsu (1992)

Bullera grandispora Derx (1930) nomen dubium sensu B.N. Johri & Bandoni (1984)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal, ovoidal, cylindroidal, sometimes somewhat constricted in the middle, and with the apices rounded or truncate, (9.0–35.0) × (5.0–12.0) µm, single or in short chains. Budding is polar with percurrent or sympodial proliferation, and with distinct annellations. Sometimes buds grow out as short hyphae. Growth is butyrous or mucoid, flat or somewhat raised, smooth, shiny, grayish-cream to pale yellowish-brown, and with the margin entire, straight or somewhat crenulate.

Growth on the surface of assimilation media (glucose): A ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, poorly developed pseudohyphae are formed. Cells are ellipsoidal, ovoidal or cylindroidal, and are sometimes rather thick-walled. Aerobic growth is pale yellowish-brown, pinkish-brown, butyrous or mucoid, flat, or somewhat raised, and smooth or somewhat reticulate in the center. The margin is entire, straight, lobate or crenulate.

Formation of ballistoconidia: Ballistoconidia, formed on morphology and corn meal agars, are bilaterally symmetrical, ovoidal, ellipsoidal or allantoid, and measure (8.0–16.0) × (4.0–9.0) µm (Fig. 382).

Fermentation: absent.

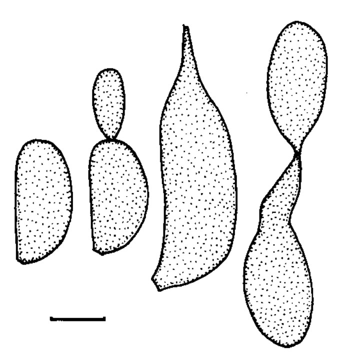


Fig. 382. *B. pyricola,* CBS 6784. Ballistoconidia on corn meal agar. Bar = 5 µm.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1986d).

Mol% G + C: 50.2–53.5, three strains, CBS 6754, CBS 6784, CBS 6982 (T_m: Boekhout 1991a); 50.8–51.7, JCM 2958, and one other strain (T_m: Nakase and Suzuki 1986c).

Origin of the strains studied: CBS 6754, from leaf of pear tree, F. Stadelmann, Switzerland; CBS 6784, from leaf of pear tree infected by *Operophthora brumata,* F. Stadelmann, Switzerland; CBS 6785, from leaf of pear tree infected by *Venturia pirina,* F. Stadelmann, Switzerland; CBS 6982, from rotten wood, originally identified as *B. grandispora* Derx, B.N. Johri and R.J. Bandoni, Canada.

Type strain: CBS 6754.

Comments: *Bullera grandispora* sensu Johri & Derx is considered conspecific with *B. pyricola,* because of about 85% DNA–DNA homology (Boekhout and Poot, unpublished results). The cell walls of CBS 6982 were found to be layered, electron-dense, and surrounded by a fibrous capsule (Boekhout, unpublished results).

93.10. *Bullera pseudoalba* Nakase & M. Suzuki (1986a)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal, (4.0–7.0)×(2.0–4.0) µm, and single. Budding is polar and buds are sessile or on short denticles. Growth is mucoid or butyrous, flat or slightly raised, smooth, shiny, brownish-yellow, and with the margin entire.

Growth on the surface of assimilation media (glucose): A ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, septate hyphae occur. Cells are ellipsoidal.

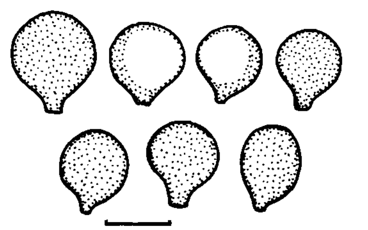


Fig. 383. *B. pseudoalba,* CBS 7227. Ballistoconidia on corn meal agar. Bar = 5 µm.

Aerobic growth is pale yellowish to pinkish yellow-brown, butyrous, shiny, flat, smooth or weakly transversely furrowed, and with the margin entire or somewhat eroded, straight or somewhat crenulate.

Formation of ballistoconidia: Ballistoconidia, formed on morphology and corn meal agars, are subglobose, and measure (5.0–10.0)×(3.5–8.0) µm (Fig. 383).

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	+
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase and Suzuki 1986a).

Mol% G + C: 52.6–53.7, two strains, JCM 5290, NB-104 (T_m: Nakase and Suzuki 1986a).

Origin of the strains studied: CBS 7227 (JCM 5281), dead leaf of rice (*Oryza sativa*), T. Nakase, Japan; CBS 7343 (JCM 5721), CBS 7344 (JCM 5722) CBS 7362 (JCM 5723), all three from dead leaves of *Miscanthus sinensis,* T. Nakase, Japan.

Type strain: JCM 5281.

Comments: *Bullera pseudoalba* is similar to *B. alba* in morphological, physiological and biochemical aspects. *Bullera pseudoalba* differs from *B. alba,* as well as from *B. oryzae* and *B. sinensis* by the assimilation of glycol (Boekhout 1991a), the formation of blastoconidia at the apex of hyphae on corn meal agar (Boekhout 1991a), more yellowish-brown colonies, and differences

in electrophoretic enzyme patterns (Nakase and Suzuki 1986a).

93.11. *Bullera punicea* (Komagata & Nakase) Nakase & M. Suzuki (1986c)

Synonyms:
Candida punicea Komagata & Nakase (1965)
Sporobolomyces puniceus (Komagata & Nakase) Ahearn & Yarrow apud Yarrow (1972)
Udeniomyces punicea (Komagata & Nakase) Nakase & Takematsu (1992)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal to cylindroidal, rarely subglobose, (10.0–17.0)×(5.5–8.5) µm, and single. Budding is polar, rarely lateral, and buds are sessile, with percurrent proliferation and scars. Cells may form hyphae of up to ca. 75 µm long and 4.0–7.0 µm wide. Growth is butyrous, somewhat raised, smooth, dull, pale pinkish-cream, and with the margin entire.

Growth on the surface of assimilation media (glucose): A weak ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, no true mycelium or pseudomycelium is formed, but short hyphal cells may be present. Cells are ellipsoidal, ogival or limoniform. Aerobic growth is cream, butyrous, shiny, flat or somewhat raised, with the margin smooth and entire.

Formation of ballistoconidia: Ballistoconidia, formed on corn meal agar, are bilaterally symmetrical, allantoid, clavate or ellipsoidal, (10.0–14.0)×(5.5–7.0) µm (Fig. 384).

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	+	Inositol	v
D-Ribose	+	Hexadecane	n
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

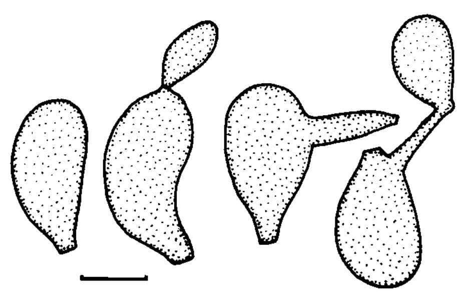


Fig. 384. *B. punicea*, CBS 5689. Ballistoconidia on corn meal agar. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
50% Glucose	–	Nitrite	+
Starch formation	+	Growth at 25°C	–

Co-Q: 10 (Nakase and Suzuki 1986d).
Mol% G+C: 53.9–54.4 (T_m: Nakase 1989), 53.3 (HPLC, Nakase unpublished results).

Origin of the strain studied: CBS 5689 (JCM 1535), frozen food, K. Komagata and T. Nakase, Japan.
Type strain: CBS 5689.

Comments: In the original diagnosis of *Candida punicea* (=*Bullera punicea*), a type was not designated. However, only one strain was listed by the original authors (Komagata and Nakase 1965). This strain, CBS 5689, was recently designated the neotype (Boekhout 1991a). Carotenoid composition, cell wall composition, and physiological characteristics were considered as indicative for affiliation of this strain with *Bullera* by Fiasson (1972), Weijman and Rodrigues de Miranda (1983) and Stadelmann (1975), respectively.

93.12. *Bullera sinensis* Li (1982)

Synonyms:
Bullera alba (Hanna) Derx var. *lactis* Li (1982)
Bullera derxii Nakase & M. Suzuki (1986a)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal, (4.5–10.0)×(3.0–5.0) µm, and single. Budding is polar, and with buds sessile or on short denticles and with percurrent or sympodial proliferation. Growth is mucoid, glossy, pale yellowish-brown, with the margin entire.

Growth on the surface of assimilation media (glucose): A thin film, islets and a sediment are formed.

Dalmau plate on morphology agar: Poorly developed pseudohyphae are formed in some strains. Cells are ellipsoidal. Aerobic growth is yellowish to pinkish-cream, mucoid, glossy, flat or with the center somewhat raised, smooth or weakly transversely striated. The margin is entire, straight or crenulate.

Formation of ballistoconidia: Ballistoconidia, formed on corn meal, potato extract and morphology agars, are flabelliform, somewhat turbinate or globose to subglobose, and measure (3.0–7.0)×(3.0–7.0) µm (Fig. 385).

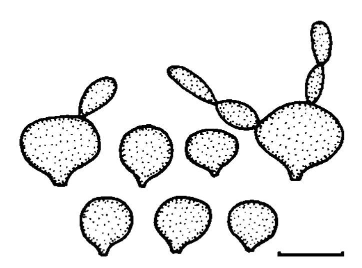


Fig. 385. *B. sinensis*, CBS 7225. Ballistoconidia on corn meal agar. Bar = 5 μm.

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	+
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase and Suzuki 1986d).

Mol% G + C: 54.5–56.8, JCM 5280 (T_m: Nakase and Suzuki 1986a), CBS 7237, CBS 7238 (T_m: Nakase et al. 1990c), 54.3–54.9 (HPLC: Nakase et al. 1990c).

Origin of the strains studied: CBS 7225 (JCM 5280), CBS 7345 (JCM 5734), CBS 7358 (JCM 5279), all three from dead leaves of rice (*Oryza sativa*), T. Nakase, Japan; CBS 7346 (JCM 5874), dead leaf of *Miscanthus sinensis*, T. Nakase, Japan; CBS 7237, from *Coccinella*, M. Li, China; CBS 7238, from leaf of wheat (*Triticum* sp.), M. Li, China.

Type strain: CBS 7238.

Comments: *Bullera sinensis* is similar to *B. alba* and can be differentiated from the latter species by lack of growth on lactose and inulin, and by the ballistoconidia which are generally somewhat broader than long. Moreover, the colony on morphology agar is slightly more pinkish. Ballistoconidia were not observed in the type strains of *B. sinensis* and *B. alba* var. *lactis*. Because of similarities in the nutritional physiology (Boekhout 1991a) and electrophoretic enzyme patterns (Nakase et al. 1990c), *B. derxii* and *B. alba* var. *lactis* are considered synonyms of *B. sinensis*.

93.13. *Bullera variabilis* Nakase & M. Suzuki (1987d)

Growth on 5% malt extract agar: After 7 days at 17°C, cells are ellipsoidal, fusiform, straight or somewhat curved, (5.0–13.5) × (3.0–5.0) μm, and single. Budding is polar or lateral, and buds are sessile or on denticles, which may be sympodially branched. Hyphal outgrowths are sometimes present. Growth is butyrous, flat or somewhat raised, smooth, shiny, pale yellowish-brown to pinkish yellow-brown, and with the margin entire, straight or somewhat undulating.

Growth on the surface of assimilation media (glucose): A ring, islets, a thin film, and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, irregularly branched pseudohyphae may be present, made up of cells measuring (15–110) × (3.0–5.5) μm. Cells have variable shapes including fusiform, ellipsoidal, allantoid or subglobose. Aerobic growth is yellowish-cream, butyrous, dull or shiny, flat or somewhat raised, smooth or weakly transversely wrinkled. The margin is smooth, straight or somewhat crenulate.

Formation of ballistoconidia: Ballistoconidia, formed on corn meal agar and potato dextrose agar, are flabelliform, transversely reniform, or somewhat turbinate, frequently somewhat angular, and measure (3.5–8.0) × (4.5–9.0) μm (Fig. 386).

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	–
Sucrose	+	Glycerol	–/l
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	+
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% Glucose	–	Growth at 25°C	+
Starch formation	+	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1987d).

Mol% G + C: 55.9–56.9, three strains, JCM 5275, NO-43, NO-11 (T_m: Nakase and Suzuki 1987d).

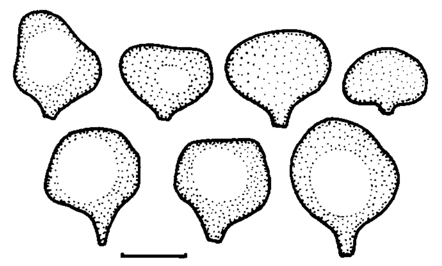


Fig. 386. *B. variabilis*, CBS 7354. Ballistoconidia on corn meal agar. Bar = 5 μm.

Origin of the strains studied: CBS 7354 (JCM 3914), dead base of bamboo culm, R.J. Bandoni, Canada; CBS 7347 (JCM 5275), dead leaf of rice (*Oryza sativa*), T. Nakase, Japan; CBS 7364 (JCM 5984), dead leaf of *Sasa* sp., T. Nakase, Japan; CBS 7365 (JCM 7265), CBS 7366 (JCM 7266), CBS 7367 (JCM 5661), all three from dead leaves of *Miscanthus sinensis,* T. Nakase, Japan.

Complementary mating types: CBS 7347 and CBS 7367.

Type strain: JCM 5275.

Comments: *Bullera variabilis* can be readily recognized by its somewhat angular ballistoconidia. Mating and dikaryon formation were observed to occur between CBS 7357 and CBS 7367 on corn meal agar. Dikaryotic hyphae are regularly septate, with clamp connections at the septa, and with cells measuring (40–120)×(2.0–3.0) μm. Cylindrical-lageniform and subglobose dikaryotic cells, measuring (7.0–27.0)×(4.0–6.5) μm, are formed laterally and terminally on the hyphae or hyphal branchlets. However, no basidia were observed. Septal pores are dolipores with parenthesomes made up of U-shaped vesicles (Boekhout et al. 1991a, Boekhout 1991a).

Comments on the genus

Derx (1930) defined *Bullera* by the rotational symmetry of the ballistoconidia. However, in the present circumscription, the genus also contains species with bilaterally symmetrical ballistoconidia. These species, viz. *B. megalospora, B. pyricola* and *B. punicea,* alternately placed in the genus *Udeniomyces* by Nakase and Takematsu (1992), are additionally characterized by the formation of annellidic scars (Boekhout 1991a). Color of the colonies, also used to differentiate *Bullera* and *Sporobolomyces,* is not a good discriminative character. *Bullera armeniaca* and *B. crocea* have orange colored colonies. Moreover, *B. alba* (= *Bulleromyces albus*) was found to contain β-carotenoids, γ-carotenoids and torulene (Fiasson 1972). Presence of xylose in whole-cell hydrolyzates is a reliable character to differentiate *Bullera* from *Sporobolomyces* (Weijman and Rodrigues de Miranda 1983, Suzuki and Nakase 1988a). Recently, it became clear that the septal pore of the dikaryophase of *Bullera* is a dolipore, with a parenthesome made up of U-shaped vesicles (Boekhout et al. 1991a). Additional characters were found in the metabolism of D-glucuronic acid (Golubev 1989b), sensitivity to killer toxins (Golubev and Kuznetsova 1989, Golubev 1990a) and nucleotide sequences of 5S rRNA (Gottschalk and Blanz 1985). The life cycle is usually heterothallic, and comprises mating of compatible yeast strains, and formation of dikaryotic hyphae and basidia in which karyogamy and meiosis occur. In addition, self-sporulating strains occur (Boekhout et al. 1991a). The presumed homothallic life cycle of *B. dendrophila* (van der Walt 1973) is not accepted, as there is no experimental evidence for a sexual cycle.

Editors' note

Based on nucleotide sequence analysis of 18S rDNA (Suh and Nakase 1995) and partial sequences of 26S rDNA (G. Scorzetti and J.W. Fell, unpublished), *Udeniomyces* is related to *Cystofilobasidium* and *Mrakia* and well separated from *Bullera.* Consequently, the separate designation of *Udeniomyces* appears correct, but the information was received too late for inclusion in this edition.

94. *Cryptococcus* Vuillemin

J.W. Fell and A. Statzell-Tallman

Diagnosis of the genus

Cells are spheroidal, ovoidal or elongate. Reproduction by multilateral or polar budding; pseudo- or true hyphae may develop. Ascospores, ballistospores and ballistoconidia are not formed. Colony color on solid media may be white or cream, and some strains produce red, yellow or brown pigments. All species utilize D-glucuronate and most species synthesize starch; those species that do not synthesize starch utilize inositol, a combination that distinguishes those species from *Rhodotorula* spp. (Table 74). Fermentative ability is lacking. Xylose is present in cell hydrolyzates. Diazonium blue B and urease reactions are positive. Coenzymes Q-9 and Q-10 are present. Some species are anamorphic states of *Filobasidium, Filobasidiella, Cystofilobasidium,* and other teleomorphic genera in the Tremellales and Filobasidiales.

Table 74
Key characteristics of the genera *Cryptococcus* and *Rhodotorula*

Starch formation	Assimilation		Tables
	Inositol	D-Glucuronate	
+	+	+	*Cryptococcus*: (1) nitrate positive, Table 76, p. 745; (2) nitrate negative, Table 77, p. 745
−	+	+	*Cryptococcus*: Table 79, p. 746
+	−	+	*Cryptococcus*: Table 78, p. 746
−	−	−	*Rhodotorula*: (1) nitrate positive, Table 90, p. 804; (2) nitrate negative, Table 91, p. 804
−	−	+	*Rhodotorula*: (1) nitrate positive, Table 88, p. 803; (2) nitrate negative, Table 89, p. 803
−	+	−	*Rhodotorula*: Table 87, p. 803

Type species

Cryptococcus neoformans (Sanfelice) Vuillemin

Species accepted

1. *Cryptococcus aerius* (Saito) Nannizzi (1927)
2. *Cryptococcus albidosimilis* Vishniac & Kurtzman (1992)
3. *Cryptococcus albidus* (Saito) C.E. Skinner (1947)
4. *Cryptococcus amylolentus* (van der Walt, D.B. Scott & van der Klift) Golubev (1981)
5. *Cryptococcus antarcticus* Vishniac & Kurtzman (1992)
6. *Cryptococcus aquaticus* (Jones & Slooff) Rodrigues de Miranda & Weijman (1988)
7. *Cryptococcus ater* (Castellani ex Cooke) Phaff & Fell (Lodder 1970)
8. *Cryptococcus bhutanensis* S. Goto & Sugiyama (1970)
9. *Cryptococcus consortionis* Vishniac (1985)
10. *Cryptococcus curvatus* (Diddens & Lodder) Golubev (1981)
11. *Cryptococcus dimennae* Fell & Phaff (1967)
12. *Cryptococcus feraegula* Saëz & Rodrigues de Miranda (1988)
13. *Cryptococcus flavus* (Saito) Phaff & Fell (1970)
14. *Cryptococcus friedmannii* Vishniac (1985)
15. *Cryptococcus fuscescens* Golubev (1984)
16. *Cryptococcus gastricus* Reiersöl & di Menna (1958)
17. *Cryptococcus gilvescens* Chernov & Bab'eva (1988)
18. *Cryptococcus heveanensis* (Groenewege) Baptist & Kurtzman (1976)
19. *Cryptococcus huempii* (Ramírez & González) Roeijmans, van Eijk & Yarrow (1989)
20. *Cryptococcus humicolus* (Daszewska) Golubev (1981)
21. *Cryptococcus hungaricus* (Zsolt) Phaff & Fell (1970)
22. *Cryptococcus kuetzingii* Fell & Phaff (1967)
23. *Cryptococcus laurentii* (Kufferath) C.E. Skinner (1950)
24. *Cryptococcus luteolus* (Saito) C.E. Skinner (1950)

25. *Cryptococcus macerans* (Frederiksen) Phaff & Fell (1970)
26. *Cryptococcus magnus* (Lodder & Kreger-van Rij) Baptist & Kurtzman (1976)
27. *Cryptococcus marinus* (van Uden & Zobell) Golubev (1981)
28. *Cryptococcus neoformans* (Sanfelice) Vuillemin (1901): see *Filobasidiella neoformans*, p. 656
29. *Cryptococcus podzolicus* (Bab'eva & Reshetova) Golubev (1981)
30. *Cryptococcus skinneri* Phaff & do Carmo-Sousa (1962)
31. *Cryptococcus terreus* di Menna (1954)
32. *Cryptococcus uniguttulatus* (Zach) Phaff & Fell (1970): see *Filobasidium uniguttulatum*, p. 667
33. *Cryptococcus vishniacii* Vishniac & Hempfling (1979)
34. *Cryptococcus yarrowii* A. Fonseca & van Uden (1991)

Key to species

See Table 75.

1.	a	Starch synthesized → 2		
	b	Starch not synthesized → 37 (Table 79)		
2(1).	a	Inositol assimilated → 3		
	b	Inositol not assimilated → 30 (Table 78)		
3(2).	a	Nitrate assimilated → 4 (Table 76)		
	b	Nitrate not assimilated → 15 (Table 77)		
4(3).	a	Sucrose assimilated → 5		
	b	Sucrose not assimilated → 13		
5(4).	a	Maltose assimilated → 6		
	b	Maltose not assimilated → 12		
6(5).	a	Colony color red → 7		
	b	Colony color not red → 10		
7(6).	a	Monokaryotic teliospores formed	*Cystofilobasidium capitatum*:	p. 648
	b	Monokaryotic teliospores not formed → 8		
8(7).	a	Erythritol assimilated	*C. macerans*:	p. 760
	b	Erythritol not assimilated → 9		
9(8).	a	Melibiose assimilated	*Cystofilobasidium bisporidii*:	p. 646
	b	Melibiose not assimilated	*Cystofilobasidium infirmominiatum*:	p. 650
10(6).	a	Teliospores formed	*Cystofilobasidium lari-marini*:	p. 652
	b	Teliospores not formed → 11		
11(10).	a	D-Glucosamine assimilated	*Filobasidium floriforme*:	p. 666
	b	D-Glucosamine not assimilated	*C. albidus*:	p. 748
			C. albidosimilis:	p. 747
			C. antarcticus:	p. 749
12(5).	a	Galactose assimilated	*Mrakia frigida*:	p. 676
	b	Galactose not assimilated	*C. kuetzingii*:	p. 759
13(4).	a	D-Glucitol assimilated → 14		
	b	D-Glucitol not assimilated	*C. fuscescens*:	p. 754
14(13).	a	*N*-Acetyl-D-glucosamine assimilated	*C. terreus*:	p. 764
	b	*N*-Acetyl-D-glucosamine not assimilated	*C. feraegula*:	p. 753
15(3).	a	Colony color red	*C. hungaricus*:	p. 758
	b	Colony color not red → 16		
16(15).	a	Maltose assimilated → 17		
	b	Maltose not assimilated → 29		
17(16).	a	Sucrose assimilated → 18		
	b	Sucrose not assimilated	*C. gastricus*:	p. 755
			C. gilvescens:	p. 756
18(17).	a	Melibiose assimilated → 19		
	b	Melibiose not assimilated → 23		
19(18).	a	Lactose assimilated → 20		
	b	Lactose not assimilated → 22		
20(19).	a	*N*-Acetyl-D-glucosamine assimilated → 21		
	b	*N*-Acetyl-D-glucosamine not assimilated	*C. laurentii*:	p. 759
21(20).	a	Erythritol assimilated	*C. humicolus*:	p. 757
	b	Erythritol not assimilated	*C. podzolicus*:	p. 762
22(19).	a	Saccharate assimilated	*C. luteolus*:	p. 760
	b	Saccharate not assimilated	*C. amylolentus*:	p. 749
23(18).	a	Lactose assimilated → 24		
	b	Lactose not assimilated → 27		
24(23).	a	Erythritol assimilated → 25		
	b	Erythritol not assimilated → 26		

Table 75
Key characters of species in the genus *Cryptococcus*

Species	Culture color[a]	Pseudohyphae[b]	Starch formation	Assimilation[c]																			
				Gal	Suc	Mal	Lac	Mel	Raf	Mlz	Xyl	Ara	Rib	Rha	Gln	*N*-A	Ery	Glu	Glo	Ino	Nit	Sac	D-G
Cryptococcus aerius	c-y/t	–	–	+	+	+	+	+	+	+	+	s	s	+	w	–	–	+	+	+	+	+	+
C. albidosimilis	w	–	+	–	+	+	+	w	–	+	+	–	–	+	–	n	–	+	+	+	+	n	+
C. albidus	c-y/t	–	+/w	v	+	+	v	v	+	+	+	v	v	v	–	–	v	+	+	+	+	+	+
C. amylolentus	w-c	rud	+	+	+	+	–	s	+	s	+	s	+	+	+	+	+	+	+	+	–	–	+
C. antarcticus	w-c	–	+	–	+	+	–	–	v	+	+	–	–	+	–	n	–	v	+	+	+	n	n
C. aquaticus	y-c	rud	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	+	+	–	+	+	+
C. ater	c-b	–	+	+/w	+	+	+	–	s	v	+	v	v	+	+	+	–	+	+	+	–	+	+
C. bhutanensis	c-bu	–	+	s	+	+	–	–	+	+	+	s	–	v	–	–	–	+	+	–	+	+	+
C. consortionis	c	–	+	–	s	–	–	–	s	–	+	–	–	s	–	–	–	–	s	–	–	–	s
C. curvatus	b/y	+	+	+	+	+/w	+	–	+	v	+	–	+	+/w	s	+	+/w	v	+	+/w	–	–	+
C. dimennae	t-pc	–	+	+	+	–	+	–	+	–	+	+	+	+	–	–	–	+	+	+	–	w	+
C. feraegula	o	rud	+	–	–	–	–	–	–	–	s	s	s	+	–	–	–	+	+	+	+	+	+
C. flavus	b-y	–	–	+	+	+	+	+	+	+	+	w	+	+	w	+	+	+	+	+	–	w	+
C. friedmannii	c	–	+	–	s	+	–	–	–	+	+	–	–	–	–	+	–	–	+	–	+	+	+
C. fuscescens	bu	–	+	v	–	–	–	–	–	–	+	–	+	–	–	w	–	–	+	+	+	+	+
C. gastricus	c/y-t	–	+	+	–	+	v	–	–	+	+	–	ws	+	–	–	–	v	s	+	–	–	+
C. gilvescens	c/y-by	–	+	+	–	+	+	–	–	+	+	–	–	v	–	n	–	s	s	+	–	n	+
C. heveanensis	c/t	–	+	+	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+	–	+	+
C. huempii	c	rud	+	+	–	+	+	–	–	s	+	s	ws	+	+	+	–	+	+	–	+	–	+
C. humicolus	y	+	+	+	+	+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	–	s	+
C. hungaricus	r-o	–	+	+	+	+	v	v	+	+	+	+/w	+/w	+	–	+	–	+	+	+	–	–	+
C. kuetzingii	c-t	–	+	–	+	–	–	–	+	–	+	w	w	–	–	–	–	+	+	+	+	w	+
C. laurentii	c-y/p-t	rud	+	+	+	+	+	+	+	+	+	+	+	+	–	–	v	+	+	+	–	+	+
C. luteolus	c-y/t-b	–	+	+	+	+	–	+	+	+	+	v	+/w	+	w	+	+	+/w	+	+	–	+	+
C. macerans	r-p	rud	+	+/w	+	+	v	–	+	+/w	+	+	v	v	–	–	+	+	s	+	+	–	+
C. magnus	c-t	–	+	w	+	+	+	–	+	+	w	v	+	v	–	–	–	+	+	+	–	+	+
C. marinus	c/b-y	+	–	–	–	–	–	–	–	–	+	s	+	+	+	+	+	+	+	+	–	–	+
Filobasidiella neoformans	w-c	–	+	+	+	+	–	–	+	+	+	+	+/w	+	+	+	v	+	+	+	–	n	+
Cryptococcus podzolicus	w-c/t	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+
C. skinneri	w-c	–	+	v	–	–	–	–	–	–	+	s	v	+	–	–	v	+	+	+	–	–	+
C. terreus	b-c/y-t	–	+	v	–	v	s	–	–	v	+	v	+	+	+	+	–	+	+	+	+	+	+
C. uniguttulatus	w-c	–	+	v	+	+	–	–	v	+	+	–	–	–	+	n	–	+	+	+	–	n	+
C. vishniacii	c	–	+	–	v	+	–	–	v	s	v	–	–	v	–	–	–	–	v	–	v	–	+
C. yarrowii	w-c	rud	–	s	+	+	–	+	+	+	s	s	–	–	+	+	–	+	+	+	+	w	+

[a] Abbreviations: b, brown; bu, buff; c, cream; o, orange; pc, pinkish-cream; r, reddish; t, tan; w, white; y, yellow.

[b] Abbreviation: rud, rudimentary pseudomycelium.

[c] Abbreviations: Gal, galactose; Suc, sucrose; Mal, maltose; Lac, lactose; Mel, melibiose; Raf, raffinose; Mlz, melezitose; Xyl, D-xylose; Ara, D-arabinose; Rib, D-ribose; Rha, L-rhamnose; Gln, D-glucosamine; *N*-A, *N*-acetyl-D-glucosamine; Ery, erythritol; Glu, D-glucitol; Glo, D-gluconate; Ino, inositol; Nit, nitrate; Sac, saccharate; D-G, D-glucuronate.

Table 76

Species of *Cryptococcus* with the following characteristics: starch formation +, inositol +, D-glucuronate +, nitrate +

Species	Starch formation	Colonies red	Teliospores[a]	Assimilation[b]										
				Ino	D-G	Nit	Gal	Suc	Mal	Mel	Gln	*N*-A	Ery	Glu
Cryptococcus albidosimilis	+	–	–	+	+	+	–	+	+	w	–	n	–	+
C. albidus	+/w	–	–	+	+	+	v	+	+	v	–	–	v	+
C. antarcticus	+	–	–	+	+	+	–	+	+	–	–	n	–	v
C. feraegula	+	+	–	+	+	+	–	–	–	–	–	–	–	+
C. fuscescens	+	–	–	+	+	+	v	–	–	–	–	w	–	–
C. kuetzingii	+	–	–	+	+	+	–	+	–	–	–	–	–	+
C. macerans	+	+	–	+	+	+	+/w	+	+	–	–	–	+	+
C. terreus	+	–	–	+	+	+	v	–	v	–	+	+	–	+
Cystofilobasidium bisporidii	+	+	het	+	+	+	+	+	+	+	v	n	–	+
C. capitatum	+	+	ss	+	+	+	+	+	+	–	–	n	–	+
C. infirmo-miniatum	+	+	het	+	+	+	+	+	+	–	–	n	–	+
C. lari-marini	+	–	ss	+	+	+	+	+	+	–	–	–	–	+
Filobasidium floriforme	+	–	het, hom	+	+	+	+	+	+	–	+	n	–	+
Mrakia frigida	+	–	ss	w/–	+	+	+	+	–	–	s	+	–	+

[a] Teliospores: hom, homothallic; het, heterothallic; ss, self-sporulating.
[b] Abbreviations: Ino, inositol; D-G, D-glucuronate; Nit, nitrate; Gal, galactose; Suc, sucrose; Mal, maltose; Mel, melibiose; Gln, D-glucosamine; *N*-A, *N*-acetyl-D-glucosamine; Ery, erythritol; Glu, D-glucitol.

Table 77

Species of *Cryptococcus* with the following characteristics: starch formation +, inositol +, D-glucuronate +, nitrate –

Species	Starch formation	Colonies red	Assimilation[a]														
			Ino	D-G	Nit	Suc	Mal	Lac	Mel	Raf	Mlz	Ara	Rha	Gln	*N*-A	Ery	Sac
Cryptococcus amylolentus	+	–	+	+	–	+	+	–	s	+	s	s	+	+	+	+	–
C. ater	+	–	+	+	–	+	+	+	–	s	v	v	+	+	+	–	+
C. curvatus	+	–	+/w	+	–	+	+/w	+	–	+	v	–	+/w	s	+	+/w	–
C. dimennae	+	–	+	+	–	+	–	+	–	+	–	+	+	–	–	–	w
C. gastricus	+	–	+	+	–	–	+	v	–	–	+	–	+	–	–	–	–
C. gilvescens	+	–	+	+	–	–	+	+	–	–	+	–	v	–	n	–	n
C. heveanensis	+	–	+	+	–	+	+	+	–	+	+	+	+	–	–	+	+
C. humicolus	+	–	+	+	–	+	+	+	+	v	+	+	+	+	+	+	s
C. hungaricus	+	+	+	+	–	+	+	v	v	+	+	+/w	+	–	+	–	–
C. laurentii	+	–	+	+	–	+	+	+	+	+	+	+	+	–	–	v	+
C. luteolus	+	–	+	+	–	+	+	–	+	+	+	v	+	w	+	+	+
C. magnus	+	–	+	+	–	+	+	+	–	+	+	v	v	–	–	–	+
C. podzolicus	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+
C. skinneri	+	–	+	+	–	–	–	–	–	–	–	s	+	–	–	v	–
Filobasidium capsuligenum	+	–	+	+	–	+	+	–	–	–	–	v	–	+	n	–	n
F. uniguttulatum	+	–	+	+	–	+	+	–	–	v	+	–	–	+	n	–	n
Filobasidiella neoformans	+	–	+	+	–	+	+	–	–	+	+	+	+	+	+	v	n

[a] Abbreviations: Ino, inositol; D-G, D-glucuronate; Nit, nitrate; Suc, sucrose; Mal, maltose; Lac, lactose; Mel, melibiose; Raf, raffinose; Mlz, melezitose; Ara, D-arabinose; Gln, D-glucosamine; *N*-A, *N*-acetyl-D-glucosamine; Ery, erythritol; Sac, saccharate.

25(24). a D-Arabinose assimilated .... *C. heveanensis*: p. 756
b D-Arabinose not assimilated .... *C. curvatus*: p. 752
26(24). a D-Glucosamine assimilated .... *C. ater*: p. 750
b D-Glucosamine not assimilated .... *C. magnus*: p. 761
27(23). a Melezitose assimilated → 28
b Melezitose not assimilated .... *Filobasidium capsuligenum*: p. 664

Table 78
Species of *Cryptococcus* with the following characteristics: starch formation +, inositol −, D-glucuronate +

Species	Starch formation	Assimilation[a]								
		Ino	D-G	Nit	Mal	Mel	Xyl	Rha	*N*-A	Glu
Cryptococcus aquaticus	+	−	+	+	+	+	+	−	−	+
C. bhutanensis	+	−	+	+	+	−	+	v	−	+
C. consortionis	+	−	+	−	−	−	+	s	−	−
C. friedmannii	+	−	+	+	+	−	+	−	+	−
C. huempii	+	−	+	+	+	−	+	+	+	+
C. vishniacii	+	−	+	v	+	−	v	v	−	−
Mrakia frigida	+	v	s	+	v	v	s	v	+	+

[a] Abbreviations: Ino, inositol; D-G, D-glucuronate; Nit, nitrate; Mal, maltose; Mel, melibiose; Xyl, D-xylose, Rha, L-rhamnose; *N*-A, *N*-acetyl-D-glucosamine; Glu, D-glucitol.

Table 79
Species of *Cryptococcus* with the following characteristics: starch formation −, inositol +, D-glucuronate +

Species	Starch formation	Assimilation[a]							
		Ino	D-G	Nit	Mal	Rib	Rha	Ery	Glu
Cryptococcus aerius	−	+	+	+	+	s	+	−	+
C. flavus	−	+	+	−	+	+	+	+	+
C. marinus	−	+	+	−	−	+	+	+	+
C. yarrowii	−	+	+	+	+	−	−	−	+
Pseudozyma tsukubaënsis	−	+	+	+	+	+	−	+	−
P. fusiformata	−	+	+	+	+	+	−	+	+
P. antarctica	−	+	+	+	+	+	+	+	+

[a] Abbreviations: Ino, inositol; D-G, D-glucuronate; Nit, nitrate; Mal, maltose; Rib, ribose; Rha, L-rhamnose; Ery, erythritol; Glu, D-glucitol.

28(27). a L-Rhamnose assimilated .... *Filobasidiella neoformans*: p. 656
b L-Rhamnose not assimilated .... *Filobasidium uniguttulatum*: p. 667
29(16). a Raffinose assimilated .... *C. dimennae*: p. 753
b Raffinose not assimilated .... *C. skinneri*: p. 763
30(2). a Maltose assimilated → 31
b Maltose not assimilated → 36
31(30). a D-Xylose assimilated → 32
b D-Xylose not assimilated .... *C. vishniacii*: p. 765
32(31). a Melibiose assimilated .... *C. aquaticus*: p. 750
b Melibiose not assimilated → 33
33(32). a *N*-Acetyl-D-glucosamine assimilated → 34
b *N*-Acetyl-D-glucosamine not assimilated → 35
34(33). a L-Rhamnose assimilated .... *C. huempii*: p. 757
b L-Rhamnose not assimilated .... *C. friedmannii*: p. 754
35(33). a D-Glucitol assimilated .... *C. bhutanensis*: p. 751
b D-Glucitol not assimilated .... *C. vishniacii*: p. 765
36(30). a Nitrate assimilated .... *Mrakia frigida*: p. 676
b Nitrate not assimilated .... *C. consortionis*: p. 751
37(1). a Inositol and D-glucuronate assimilated → 38
b Inositol or D-glucuronate assimilated or both not assimilated .... *Rhodotorula*: p. 800
38(37). a Nitrate assimilated → 39
b Nitrate not assimilated → 43
39(38). a Erythritol assimilated → 40
b Erythritol not assimilated → 42
40(39). a L-Rhamnose assimilated .... *Pseudozyma antarctica*: p. 791
b L-Rhamnose not assimilated → 41
41(40). a D-Glucitol assimilated .... *Pseudozyma fusiformata*: p. 793
b D-Glucitol not assimilated .... *Pseudozyma tsukubaënsis*: p. 795
42(39). a D-Ribose assimilated .... *C. aerius*: p. 747
b D-Ribose not assimilated .... *C. yarrowii*: p. 765
43(38). a Maltose assimilated .... *C. flavus*: p. 754
b Maltose not assimilated .... *C. marinus*: p. 762

Systematic discussion of the species

94.1. *Cryptococcus aerius* (Saito) Nannizzi (Pollacci & Nannizzi 1927)

Synonyms:
Torula aeria Saito (1922)
Torulopsis aeria (Saito) Lodder (1934)
Paratorulopsis aeria (Saito) Novák & Zsolt (1961)
Cryptococcus albidus (Saito) C.E. Skinner var. *aerius* (Saito) Phaff & Fell (1970)

Growth in malt extract: After 3 days at 20°C, the cells are globose to ovoidal, (3.4–9.4)×(4.0–10.1) μm and may be single, in pairs and often in small clusters or in short chains. Usually a small amount of sediment is formed. After one month a moderate ring, no film or a few moist islands and a moderate to heavy sediment are present.

Growth on malt agar: After one month at 20°C, the streak culture is cream-colored to slightly yellowish or buff, mostly smooth, highly glossy, soft, with the margin entire or sometimes rough and wrinkled with an irregular margin.

Slide culture on potato agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	s
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	s	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	+	Citrate	+
D-Arabinose	s	Inositol	+
D-Ribose	s	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	w	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	v	Gelatin liquefaction	n
Saccharate	+	Urease	+
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Yamada and Kondo 1972a).
Mol% G+C: 55.5 (T_m: Nakase and Komagata 1968b); 54.3 (T_m: Sugita et al. 1992).
Cell hydrolyzates: Not determined.

Origin of the strain studied: Air in Tokyo (1).

Type strain: CBS 155, isolated by Saito from the air.

Comments: *Cryptococcus aerius* has been considered a variety of *C. albidus* (Rodrigues de Miranda 1984d), however, LSU rDNA nucleotide sequence analysis in our laboratory (unpublished) demonstrated that the two taxa are phylogenetically distinct and separate species. The two species can be separated by abilities to produce starch and grow in vitamin-free media.

94.2. *Cryptococcus albidosimilis* Vishniac & Kurtzman (1992)

Growth in 1% glucose–yeast extract–peptone water: The cells are ovoid, encapsulated and functionally monopolar. The organisms slowly produce a scanty annulus and a little sediment.

Growth on 1% glucose–yeast extract–peptone agar: The colony is mucoid and shining white. The average size of the cells is 6.6×4.9 μm. Neither mycelium nor pseudomycelium is formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	–	Ethanol	+/w
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	w/–	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	w	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	n	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	n	Ethylamine	–
Saccharate	n	Starch formation	+
D-Glucuronate	+	Urease	n
Xylitol	w/–	Gelatin liquefaction	n
L-Arabinitol	–	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	n	0.01% Cycloheximide	–
		Growth at 25°C	+
10% NaCl/5% glucose	n	Growth at 30°C	s
Cadaverine	+	Growth at 37°C	–
Creatine	–		

Co-Q: Not determined.
Mol% G+C: 55 (BD: Vishniac and Kurtzman 1992).
Cell hydrolyzates: Not determined.

Origin of the strain studied: Soil in Antarctica (Vishniac and Kurtzman 1992).

Type strain: CBS 7711, isolated from soil from Linnaeus Terrace, Wright Valley, South Victoria Land, Antarctica (Vishniac strain MYSW A823-2Y761/70).

Comments: This species came belatedly to our attention, therefore the above information is from the original

description (Vishniac and Kurtzman 1992). According to this description, *C. albidosimilis* is indistinguishable from *C. albidus*. The authors described this strain as a new species based on a low (18%) DNA reassociation between the type strain of *C. albidosimilis* and *C. albidus*. Based on mol% G+C contents (50–55%), *C. albidus* consists of a complex of species that is possibly reflected in the list of synonyms of *C. albidus*. The relationship of *C. albidosimilis* to these synonyms must be determined. Analysis of LSU rDNA nucleotide sequences in our laboratory (unpublished) does, however, confirm that the type strains of *C. albidosimilis* and *C. albidus* represent separate species, consistent with the reported low nDNA relatedness.

94.3. *Cryptococcus albidus* (Saito) C.E. Skinner (1947b)

Synonyms:
Torula albida Saito (1922)
Torulopsis albida (Saito) Lodder (1934)
Torulopsis albida (Saito) Lodder var. *japonica* Lodder (1934)
Torula gelatinosa Saito (1922)
Rhodotorula gelatinosa (Saito) Hasegawa, Banno & Yamauchi (1960)
Torulopsis rotundata Redaelli (1925)
Torulopsis liquefaciens Saito & Oda (1934)
Torulopsis nadaensis Saito & Oda (1934)
Torulopsis diffluens Zach (Wolfram and Zach 1934b)
Cryptococcus diffluens (Zach) Lodder & Kreger-van Rij (1952)
Rhodotorula diffluens (Zach) Hasegawa, Banno & Yamauchi (1960)
Cryptococcus albidus (Saito) C.E. Skinner var. *diffluens* (Zach) Phaff & Fell (1970)
Cryptococcus diffluens Zach var. *urugaiensis* Artagaveytia-Allende & Aciole de Queiroz (1970)
Torulopsis acris var. *granulosa* Marcilla & Feduchy (Lodder 1970) nom. dub.
Torula alpina (Grüss) Lodder (1934) nom. nud.
Rhodotorula alpina (Grüss) Krasil'nikov (1954b) nom. nud.
Hansenula amylofaciens Dietrichson (1954) nom. nud.
Cryptococcus mucorugosus Benham (1955) nom. nud.
Cryptococcus neoformans (Sanfelice) Vuillemin var. *innocuous* Benham (1955) nom. nud.
Torulopsis dattila (Kluyver) Lodder var. *armeniaca* Sarukhanyan (1957)
Torulopsis dattila (Kluyver) Lodder var. *armeniensis* Sarukhanyan (1957)
Torulopsis pseudoaeria Zsolt (1958)
Paratorulopsis pseudoaeria (Zsolt) Novák & Zsolt (1961)
Cryptococcus terricolus Pedersen (1958)
Cryptococcus genitalis Castellani (1963) nom. nud.
Naganishia globosa S. Goto (1963)
Cryptococcus albidus (Saito) C.E. Skinner var. *ovalis* Sugiyama & Goto (1967)
Torulopsis ptarmiganii Hedrick nom. nud.? (Barnett et al. 1990)

Growth in malt extract: After 3 days at 25°C, the cells are globose to ovoidal and single, in pairs and often in small clusters or short chains; elongate cells may be present. Some strains have small cells, e.g., (3.0–6.1)×(3.5–6.2) μm, other strains have larger cells, e.g., (3.5–8.8)×(5.5–10.2) μm to 14 μm long. Usually a small amount of sediment is formed. After one month a moderate ring, no film or a few moist islands and a moderate to heavy sediment are present.

Growth on malt agar: After one month at 25°C, the streak culture is cream-colored to slightly yellowish or tan, mostly smooth, highly glossy, soft and slimy with the margin entire or sometimes rough and wrinkled with an irregular margin.

Slide culture on potato agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+/w
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	v
Trehalose	+/w	Galactitol	v
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+/w
5-Keto-D-gluconate	v	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: 10 (Yamada and Kondo 1972a).
Mol% G+C: 53.7, *C. albidus*, type strain, CBS 142 (T_m: Nakase and Komagata 1971c); 55 (BD: Storck et al. 1969); 54.1–55.1, *C. diffluens*, type strain, CBS 160; 50.5, *Naganishia globosa*, type strain, CBS 5106 (T_m: Nakase and Komagata 1971c).
Cell hydrolyzates: Xylose, mannose low, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Air (4), bumble bee (1), skin of dog (1), sputum, lung and bronchial secretion (7), patients with various diseases (4), nails (3), skin (1), sake moto (2), wine (2), dew-retted flax (2), leaves (2), soil (1), blue cheese (*Naganishia globosa*) (1), goat dung (1), washed bottle in brewery (1), purification tank for polluted water (1), unknown (4), a strain labeled *Torulopsis dattila* var. *armeniensis* of unknown origin.

Type strain: CBS 142, isolated by Saito from the air.

Comments: As indicated by the variability in mol% G+C, *C. albidus* consists of a complex of species. This variability is also reflected in the large number of synonyms. Sugita et al. (1992) reported that the variety *diffluens* is a separate species based on limited DNA relatedness (41%) with *C. albidus*. This observation has been confirmed by LSU rDNA nucleotide sequence analysis in

our laboratory. Similarly, we find that *C. terricolus* is a separate species. These unpublished data were obtained after the completion of this manuscript.

94.4. *Cryptococcus amylolentus* (van der Walt, D.B. Scott & van der Klift) Golubev (1981)

Synonym:
Candida amylolenta van der Walt, D.B. Scott & van der Klift (1972)

Growth in 1% glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are spheroidal, ovoidal or ellipsoidal, (3.4–6.7)×(3.4–7.4) µm. After one month there is a sediment, a ring and a few islets.

Growth on 1% glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is white to cream-colored, pasty, dull and smooth. The margin is entire to undulating.

Dalmau plate culture on corn meal agar: The pseudomycelium consists of ramified chains of ovoidal to elongate cells, bearing clusters of short, ovoidal blastoconidia in verticillate positions.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	s	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	ws
Melezitose	s	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	s	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	ws
50% (w/w) glucose–yeast extract agar	+	Growth at 37°C	–
10% NaCl/5% glucose	w		

Co-Q: Not determined.
Mol% G+C: 55.1, type strain (T_m: Meyer et al. 1984).
Cell hydrolyzates: Xylose, mannose low, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Frass from tunnels of the beetle *Enneadesmus forficulus* Fairm. in timber of *Dombeya rotundifolia* (Hochst.) Harv. near Grobersdal in the Transvaal, South Africa (1); frass from the tunnels of *Sinoxylon ruficorne* Fahr. infesting *Dichrostachys cinerea* L. (Wright & Arn.) near Grobersdal in the Transvaal (1) (van der Walt 1972).

Type strain: CBS 6039, from frass of the tunnels of *Enneadesmus forficulus*.

Comments: Nucleotide sequence analysis of a region of the LSU rDNA indicates that *C. amylolentus* is related to *Tsuchiyaea wingfieldii* (Fell et al. 1995).

94.5. *Cryptococcus antarcticus* Vishniac & Kurtzman (1992)

Growth in 1% glucose–yeast extract–peptone water: A scanty annulus and a little sediment are produced. The cells are ovoid, possess a scanty capsule and are functionally monopolar, budding repeatedly from the site of the primary bud scar.

Growth on 1% glucose–yeast extract–peptone agar: The colony is slightly mucoid and shining white to cream. The cells measure 4.0–7.5 µm. Neither mycelium nor pseudomycelium is formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	n	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	50% (w/w) glucose–yeast extract agar	n
5-Keto-D-gluconate	n	10% NaCl/5% glucose	n
Saccharate	n	Starch formation	+
D-Glucuronate	n	Urease	n
Xylitol	–	Gelatin liquefaction	n
L-Arabinitol	–	0.01% Cycloheximide	–
Cadaverine	+	Growth at 15°C	+
Creatine	–	Growth at 20°C	v
L-Lysine	+	Growth at 30°C	–
Ethylamine	v		

Co-Q: Not determined.
Mol% G+C: 55 (T_m: Vishniac and Kurtzman 1992).
Cell hydrolyzates: Not determined.

Origin of the strain studied: Soil in Antarctica (Vishniac and Kurtzman 1992).

Type strain: CBS 7687, isolated from soil from University Valley, South Victoria Land, Antarctica (Vishniac strain MYSW A812-20bY693/64).

Comments: Because this species came belatedly to our attention, the above descriptive information is from the original description (Vishniac and Kurtzman 1992). According to that description, *C. antarcticus* is phenotypically indistinguishable from *C. albidus* and the species distinction is based on a low (14%) DNA reassociation between the type strains of *C. antarcticus* and *C. albidus*. Analysis of LSU rDNA nucleotide sequences in our laboratory (unpublished) confirms that *C. albidus* and *C. antarcticus* are separate species and that *C. antarcticus* is closely related to *C. bhutanensis*.

94.6. *Cryptococcus aquaticus* (Jones & Slooff) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonyms:
Candida aquatica Jones & Slooff (1966)
Vanrija aquatica (Jones & Slooff) R.T. Moore (1980)

Growth in glucose–yeast extract–peptone water: After 3 days at 19°C, the cells are ovoidal or elongate, (2.7–5.4)×(8.0–13.4) µm. Budding is mono- or bipolar; multiple buds, which form on separate sites at the poles, remain attached to the parent cells. Groups of cells thus formed have a star-like arrangement. After one month a thin ring and a heavy sediment are present.

Growth on glucose–yeast extract–peptone agar: After one month at 19°C, the streak culture is yellowish cream-colored, semi-dull, soft, and slightly wrinkled.

Dalmau plate culture on corn meal agar: The pseudomycelium consists of branched short chains of ovoidal or elongate cells. The elongate cells may be flask-, dumbbell- or club-shaped, lageniform or display other unusual shapes. (Fig. 387).

Fermentation:

Glucose	w	Maltose	v
Galactose	–	Lactose	–
Sucrose	v	Raffinose	v

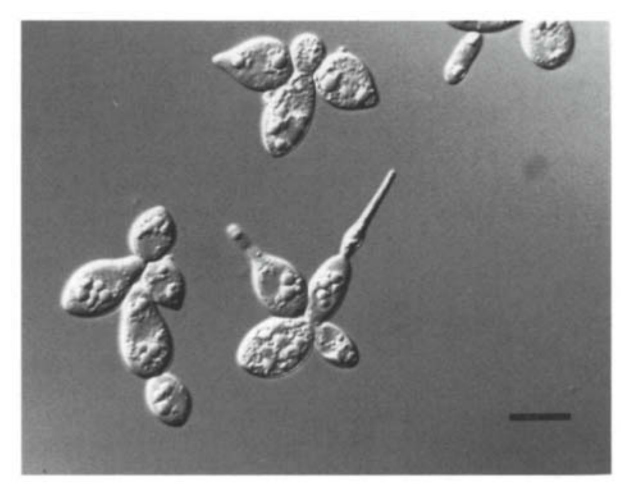


Fig. 387. *C. aquaticus*, CBS 6758. Cells grown on corn meal agar at 19°C for 2 weeks. Bar = 10 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	+	Citrate	s
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Urease	+
5-Keto-D-gluconate	n	Gelatin liquefaction	–
Saccharate	+	Biotin-free	–
D-Glucoronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 25°C	+
10% NaCl/5% glucose	–	Growth at 30°C	–
Starch formation	+		

Co-Q: Not determined.
Mol% G + C: 58.4, type strain (BD: Meyer et al. 1984).
Cell hydrolyzates: Fucose, xylose, mannose low (Weijman and Rodrigues de Miranda 1988)

Origin of the strain studied: Scum on a freshwater mountain lake in Malham Tarn, Yorkshire, England, Oct. 1962 (Jones and Slooff 1966).

Type strain: CBS 5443 (Jones and Slooff strain P.181).

Comments: Jones and Slooff (1966) found this species in "large quantities" in water scums in Oct. 1962 and March 1963 in one area, Malham Tarn. They did not find the fungus in scums collected in other parts of Great Britain. They speculated the presence or absence of the yeast was dependent on the types and levels of organic material in the water. Based on morphological and physiological criteria, Jones and Slooff (1966) noted the possible relationship of *C. aquaticus* to species currently included in the genus *Mrakia*. This observation is in agreement with results of nucleotide sequence alignment comparisons of a region the LSU rDNA (Fell et al. 1995).

94.7. *Cryptococcus ater* (Castellani ex Cooke) Phaff & Fell (Lodder 1970)

Synonyms:
Cryptococcus ater Castellani (1960) nom. nud.
Cryptococcus laurentii (Kufferath) C.E. Skinner var. *magnus* Lodder & Kreger-van Rij forma *ater* Castellani ex Cooke (1966)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to globose, (3.7–6.9)×(4.0–8.0) µm, rarely to 9 µm long; single or in pairs, a few short chains

of three and four cells. A thin ring and a little sediment are present. After one month there is a moderate ring and a thick sediment.

Growth on 5% malt agar: After one month, the streak culture is cream to buff to blackish-brown, smooth, glossy, slimy (running to the bottom of the tube). The dark color infuses into the agar.

Dalmau culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+/w	Methanol	–
L-Sorbose	–/l	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	s	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+/w
D-Arabinose	v	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Roeymans and Zijlstra, cited by Guého et al. 1993).
Mol% G+C: 48.6 (PC: Golubev and Vagabova 1977).
Cell hydrolyzates: Xylose, mannose dominate (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Ulcers on leg of a patient (3).

Type strain: CBS 4685, the original strain of Castellani (1960) isolated from multiple ulcers on the leg of a young man.

Comments: Based on molecular sequence analyses, *C. ater* may be closely related to *Filobasidium elegans* (Guého et al. 1993) and *C. magnus* (Yamada et al. 1990a). Sequence analyses in our laboratory confirm close relationships of *C. ater, C. magnus, F. elegans* and *F. floriforme.*

94.8. *Cryptococcus bhutanensis* S. Goto & Sugiyama (1970)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to spheroidal, measuring (4.0–6.7×4.7–8) µm and may be single or budding. After one month a sediment is present.

Growth on 5% malt agar: After one month at 25°C, the streak culture is cream to buff-colored, smooth, slightly glistening and the edges are entire.

Dalmau plate culture on corn meal agar: Both true and pseudomycelium are absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	s	Methanol	–
L-Sorbose	+	Ethanol	v
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	w	D-Gluconate	+
Soluble starch	w	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	+	Citrate	s
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	w/–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 53.4 (T_m: Nakase and Komagata 1971c); 54.2 (BD: Baharaeen et al. 1982).
Cell hydrolyzates: Xylose, mannose dominate (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Soil sample from Laya, Bhutan, collected May 1967 (Goto and Sugiyama 1970).

Type strain: CBS 6294 (Goto and Sugiyama strain H-3-9-3, designated as type no. TI-0029).

Comments: *C. bhutanensis,* based on unpublished nucleotide sequence data in our laboratory, is related to *C. antarcticus.*

94.9. *Cryptococcus consortionis* Vishniac (1985a)

Growth in 5% malt extract: After 3 days at 19°C, the cells are ovoidal to spherical, (3.4–6.0)×(4.0–6.7) µm, single or in pairs. Budding is polar. After one month there is little sediment but neither a ring, nor a pellicle are present.

Growth on 5% malt extract agar: After one month at 19°C, the streak colony is cream-colored, flat and semi-glistening.

Dalmau plate culture on corn meal agar: After one week at 19°C, pseudomycelium is not formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	s	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	s	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	s
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	s	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Thiamine-free	–
D-Glucuronate	s	Growth at 19°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 23°C	s
		Growth at 27°C	–
10% NaCl/5% glucose	–		
Starch formation	+		

Co-Q: Not determined.
Mol% G+C: 56 (T_m: Vishniac 1985a).
Cell hydrolyzates: Not determined.

Origin of the strain studied: Soil from the dry valleys of South Victoria Land, Antarctica.

Type strain: CBS 7159 (Vishniac strain MYSW A 801-3aY92).

Comments: Contrary to the original description, we found this strain to produce urease and to utilize raffinose, L-rhamnose, mannitol and D-gluconate.

94.10. *Cryptococcus curvatus* (Diddens & Lodder) Golubev (1981)

Synonyms:
Candida heveanensis (Groenewege) Diddens & Lodder var. *curvata* Diddens & Lodder (1942)
Candida curvata (Diddens & Lodder) Lodder & Kreger-van Rij (1952)
Azymocandida curvata (Diddens & Lodder) Novák & Zsolt (1961)
Apiotrichum curvatum (Diddens & Lodder) von Arx & Weijman (1979)
Vanrija curvata (Diddens & Lodder) R.T. Moore (1980)

Growth on 1% glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is either brownish, mucoid, soft and smooth or yellowish, moist to dull, soft and wrinkled.

Growth in 1% glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal to sausage-shaped, often curved, (2.7–4.7)×(3.3–9.4) μm; many cells show lateral budding on a rather broad base. A thin, dull, creeping pellicle or a thick ring is usually formed, as well as a heavy sediment.

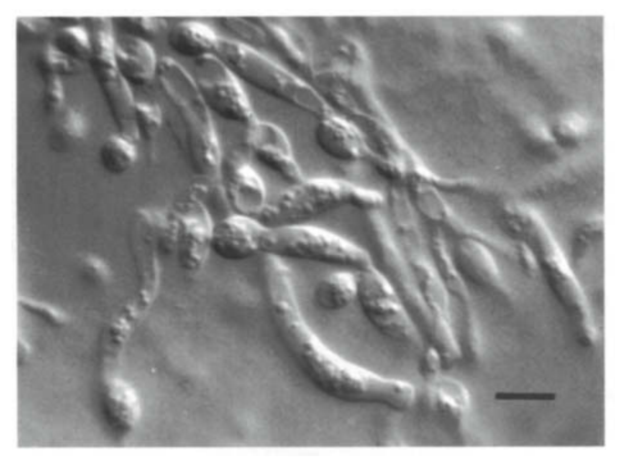


Fig. 388. *C. curvatus*, CBS 570. Cells grown on corn meal agar at 25°C for 10 days. Bar = 5 μm.

Dalmau plate culture on corn meal agar: The pseudomycelium consists either of branched chains of short pseudohyphae or long, wavy pseudohyphae bearing a few blastospores (Fig. 388).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+/w
Sucrose	+	Glycerol	+
Maltose	+/w	Erythritol	+/w
Cellobiose	+	Ribitol	+/w
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	v
D-Xylose	+	Succinate	v
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	+/w
D-Ribose	+	Hexadecane	–
L-Rhamnose	+/w	Nitrate	–
D-Glucosamine	s	Vitamin-free	w/–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Urease	+
5-Keto-D-gluconate	n	Gelatin liquefaction	–
Saccharate	–	Biotin-free	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	–
10% NaCl/5% glucose	–		
Starch formation	+		

Co-Q: 10 (Yamada and Kondo 1972a).
Mol% G+C: 59.8–60.3, 2 strains (T_m: Nakase and Komagata 1971c); 54.2, type strain (T_m: von Arx and Weijman 1979).
Cell hydrolyzates: Xylose, mannose low, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Human sputum.

Type strain: CBS 570, isolated in 1932 in the Netherlands from sputum of a patient with tuberculosis (Diddens and Lodder 1942).

Comments: Other strains have been isolated from human sputum (1), human feces (1), human urine (2), tissue culture medium (1), uterus of a cow (1) and uncertain origin (3). Nakase and Komagata (1971c) reported the species from frozen food. Nucleotide sequence analysis of the LSU rRNA indicates that *C. curvatus* is related to the genus *Trichosporon* (Guého et al. 1993).

94.11. *Cryptococcus dimennae* Fell & Phaff (1967)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal, occasionally spheroidal, (3.3–6.8)×(6.0–11.4) μm and may be single or in pairs. No ring or pellicle and very little sediment form. After one month there is a thin ring, a thin, smooth pellicle and a moderate amount of sediment.

Growth on 5% malt agar: After one month at 25°C, the streak culture is tan to pinkish-cream-colored; the surface is smooth and glossy, the texture soft and the margin entire.

Dalmau culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	ws	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	s
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Urease	+
Saccharate	w	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 9 (Roeymans and Zijlstra, cited by Guého et al. 1993).
Mol% G+C: 53.1 (T_m: Nakase and Komagata 1971c).
Cell hydrolyzates: Xylose, mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Isolated by M.E. di Menna in 1960 from leaves of pasture plants at Hankes Bay, New Zealand (2).

Type strain: CBS 5770 (di Menna strain number 60 BGF 10).

Comments: M.E. di Menna (personal communication) reported the isolation of two strains of this species from pasture plants, but she was unable to isolate additional strains from the same habitat during repeated samplings over a 3–4 year period.

94.12. *Cryptococcus feraegula* Saëz & Rodrigues de Miranda (1988)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to spherical, measuring (3–7)×(5–8) μm, and they may be budding or single. After one month a thin ring and a slight sediment are present.

Growth on 5% malt agar: After one month at 25°C, the streak culture is orange, smooth, glistening, slightly raised and the margin is entire.

Dalmau plate culture on corn meal agar: After 7 days at 19°C, short pseudohyphae are formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	s	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	s	Inositol	+
D-Ribose	s	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: Not determined.
Cell hydrolyzates: Not determined.

Origin of the strains studied: CBS 7201, from the mouth of a rhea (*Rhea americana*) that was born in captivity and died in Oct. 1978 in the Zoological Park Museum, Paris, France (Saëz and Rodrigues de Miranda 1988).

Type strain: CBS 7202 (Saëz and Rodrigues de Miranda No A3539 (B)) from a male baboon (*Papio papio*) that died in Nov. 1976 in the Zoological Park of Paris (Saëz and Rodrigues de Miranda 1988).

Comments: In addition to the strains listed above, Saëz and Rodrigues de Miranda (1988) isolated a strain from a female aye-aye (*Daubentonia madagascariensis*) in the same zoo in April 1986. Sequence analysis in our laboratory indicates that *C. feraegula* is related to *Cystofilobasidium.*

94.13. *Cryptococcus flavus* (Saito) Phaff & Fell (1970)

Synonyms:
Torula flava Saito (1922)
Chromotorula flava (Saito) F.C. Harrison (1928)
Rhodotorula flava (Saito) Lodder (1934)
Rhodotorula tokyoensis Kobayoshi ex Hasegawa var. *flava* (Saito) Hasegawa (1958)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal or irregular; single or in pairs, (3.2–5.2)×(5.0–8.8) µm. A thin ring and a little sediment begin to form. After one month a moderate yellowish ring and sediment are present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is light brown-yellow, semiglossy and nearly smooth with a butyrous texture and entire margins.

Dalmau plate culture on corn meal agar: No pseudomycelium is present, although a few chains of cells may be formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	w
Trehalose	+	Galactitol	w
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	w	D-Gluconate	+
Soluble starch	+	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w
D-Arabinose	w	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	w	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Urease	+
Saccharate	w	Gelatin liquefaction	w
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 10 (Yamada and Kondo 1973).
Mol% G+C: 55.0 (BD: Storck et al. 1969).
Cell hydrolyzates: Xylose, mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Isolated by Saito from the air in Tokyo (Saito 1922).

Type strain: CBS 331.

94.14. *Cryptococcus friedmannii* Vishniac (1985b)

Growth in 5% malt extract: After 3 days at 19°C, the cells are ovoidal, (3.4–8.0)×(4.7–11.4) µm. After one month a sediment is present.

Growth on 5% malt extract agar: After one month at 19°C, the streak culture is cream-colored, semi-glistening, smooth, flat with edges entire.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	s	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	s	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	s
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	w
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 25°C	s
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Vishniac 1985b).
Mol% G+C: Not determined.
Cell hydrolyzates: Not determined.

Origin of the strain studied: A single strain was isolated from an Antarctic rock bearing a cryptoendolithic lichen (Vishniac strain MYSW A801-133Y100).

Type strain: CBS 7160.

Comments: We found that the production of urease was weak rather than negative as reported in the original description. Analysis of the nucleotide sequence of the LSU rRNA (Fell et al. 1992) confirmed that *C. friedmannii* and *C. vishniacii* are separate species.

94.15. *Cryptococcus fuscescens* Golubev (1984)

Growth in 5% malt extract: After 3 days at 25°C, the cells are spheroidal, measuring (5.4–7.4)×(5.4–8.0) µm, and they occur singly and in pairs. After one month a sediment is present.

Growth on 5% malt extract agar: After one month at 25°C, the streak culture is buff, flat, semi-glistening, butyrous and the margin is entire.

Dalmau plate culture on corn meal agar: After one month at 25°C, no pseudomycelium is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	w
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	s	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	s
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	+	Citrate	s
D-Arabinose	–	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 25°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G+C: 52.4 (PC: Golubev 1984).

Cell hydrolyzates: Glucose and glucosamine predominant (Golubev 1984).

Monosaccharide composition of extracellular polysaccharides: Glucose, mannose, xylose, galactose and glucuronic acid (Golubev 1984).

Origin of the strain studied: Isolated by Bab'eva in 1962 from saline Takyr soil from the valley of the river Axai, Murgabski region, East Pamir, USSR.

Type strain: CBS 7189 (Golubev type MGU 674; Acad. Sci. USSR strain BKM Y-2600).

Comments: Golubev (1984) reported in the original description that *C. fuscescens* is related to *C. terreus,* based on the brown pigmentation on old malt agar cultures, positive assimilation of D-ribose, and the inability to assimilate α-methyl-D-glucoside, sucrose and raffinose. These characteristics distinguish these two species from the other nitrate-positive cryptococcoid species. This close relationship was also observed in the nucleotide sequence analysis of the LSU rRNA of each species (Fell et al. 1992). *C. fuscescens* differs from *C. terreus* by the inability to assimilate L-sorbose, lactose, L-rhamnose, D-glucosamine, D-glucitol and the inability to grow in vitamin-free medium.

94.16. *Cryptococcus gastricus* Reiersöl & di Menna (1958)

Growth in 5% malt extract: After 3 days at 20°C, the cells are mainly globose, but also short-ovoidal, (3.3–10.0)×(4.0–10.0) µm; single, in pairs or in short chains. After one month at 20°C, there is a moderate ring and a small amount of sediment.

Growth on 5% malt agar: After one month at 20°C, the streak culture is pale, cream or yellow to tan-colored. The surface is smooth and glossy, the texture slightly mucoid. The growth is flat to low convex with an irregular to entire margin.

Dalmau plate culture on corn meal agar: Pseudomycelium absent; a few chains of cells may be formed under the coverslip.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	–	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	s
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	w/–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	s	DL-Lactate	–
D-Xylose	+	Succinate	+/w
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	+
D-Ribose	ws	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	w	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 20°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G+C: 51.0, strain CBS 1927; 65.5, strain CBS 2288 (BD: Storck et al. 1969).

Cell hydrolyzates: Xylose, mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Soil in New Zealand (1) received from di Menna; washings of the stomach of a tuberculosis patient in Norway (1).

Type strain: CBS 2288 (Reiersöl and di Menna strain V39) from stomach lavage of a tuberculosis patient in Norway.

Comments: Because Reiersöl and di Menna (1958) did not designate a type strain, Phaff and Fell (1970) designated CBS 1927 as the type culture; that strain was isolated by di Menna from soil. This designation has been followed by subsequent authors (Rodrigues de Miranda 1984d, Barnett et al. 1990) and it is listed as the type culture in the CBS and ATCC cataloges. However, the

Reiersöl and di Menna (1958) description is based on strain V39 and the statement is made "In this genus V39, according to its assimilation pattern, represents a new species". The only mention of soil isolates is: "the same species was earlier isolated in New Zealand by one of the authors (di Menna) from a soil near Dunedin. She recovered a total of six strains of the species". It is clear that the authors intended V39 to be the type strain of *C. gastricus.* The reported strain differences in mol% G+C values suggest that the species may be genetically heterogenous and represent more than one species. In contrast to those results, our nucleotide sequence analyses (unpublished) indicate that the two strains are identical. In light of any potential differences, the name *C. gastricus* should be retained for strain V39 (CBS 2288).

94.17. *Cryptococcus gilvescens* Chernov & Bab'eva (1988)

Growth in 5% malt extract: Not determined.

Growth on 5% malt agar: Not determined.

Dalmau plate culture on corn meal agar: Not determined.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	s
Sucrose	–	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	n
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	n
5-Keto-D-gluconate	–	Gelatin liquefaction	n
Saccharate	n	Butanediol	–
D-Glucuronate	+	L-Malic acid	n
Arbutin	s	L-Tartaric acid	n
Xylitol	–	Sodium nitrate	n
L-Arabinitol	–	Glucono-δ-lactone	+
Cadaverine	+	D-Galacturonic acid	–
Creatinine	–	Propanediol	–
Creatine	–	Imidazole	–
L-Lysine	+	0.01% Cycloheximide	s
50% (w/w) glucose–yeast extract agar	n	0.1% Cycloheximide	n
		Growth at 25°C	+
10% NaCl/5% glucose	n	Growth at 30°C	+
Starch formation	n		

Co-Q: 9 (Chernov and Bab'eva 1988).
Mol% G+C: Not determined.
Cell hydrolyzates: Not determined.

Origin of the strain studied: Humus from tundra, western Taimyr near Dikson, Russia.

Type strain: CBS 7525.

Comments: This species was brought to our attention after the completion of this manuscript. The data above were provided by D. Yarrow, Centraalbureau voor Schimmelcultures.

94.18. *Cryptococcus heveanensis* (Groenewege) Baptist & Kurtzman (1976)

Synonyms:
Torula heveanensis Groenewege (1921b)
Candida heveanensis (Groenewege) Diddens & Lodder (1942)
Torulopsis heveanensis (Groenewege) Mager & Aschner (1947)
Cryptococcus laurentii (Kufferath) C.E. Skinner var. *magnus* Lodder & Kreger-van Rij (1952)
Cryptococcus magnus (Lodder & Kreger-van Rij) Baptist & Kurtzman var. *heveanensis* (Groenewege) Golubev (1981)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to elongate, and single, in pairs or in short chains of three or four cells, (2.0–4.5)×(3.0–7.0) µm. A small ring and some sediment are present. After one month a moderate thick ring and a heavy sediment are formed.

Growth on 5% malt agar: After one month the streak culture is smooth, glossy, cream-colored with a tan line in the middle; it is mucoid and the growth runs to the bottom of the slant. The margin is entire.

Dalmau plate culture on corn meal agar: Short chains of cells form.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	–
10% NaCl/5% glucose	w		

Co-Q: Not determined.
Mol% G+C: 49.0 (BD: Storck et al. 1969); 52.0 (BD: Baptist and Kurtzman 1976).
Cell hydrolyzates: Xylose, mannose low, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Rubber (1).

Type strain: CBS 569, isolated by Groenewege (1921b) from rubber stored in a moist room.

Comments: This species was originally described in the genus *Torula* by Groenewege (1921b). Subsequently, based on the presence of primitive pseudomycelium Diddens and Lodder (1942) transferred the species to the genus *Candida*. Mager and Aschner (1947) found that the species produced "starch" and then transferred the species to *Torulopsis*. Lodder and Kreger-van Rij (1952) considered the species as a synonym of *C. laurentii* var. *laurentii*. Phaff and Fell (1970) transferred the species to synonymy with *C. laurentii* var. *magnus*. Baptist and Kurtzman (1976) established *Cryptococcus heveanensis* based on differences in mol% G+C (*C. laurentii* var. *laurentii*, 59.7; *C. laurentii* var. *magnus*, 54.1; *C. heveanensis*, 52.0) and by differences in electrophoretic enzyme patterns. Nucleotide sequence analysis in our laboratory (unpublished) confirmed that these three species are distinct. *C. heveanensis* can be separated from *C. laurentii* var. *laurentii* by the inability to assimilate melibiose and from *C. magnus* by the ability of *C. heveanensis* to assimilate L-rhamnose, erythritol and ribitol.

94.19. *Cryptococcus huempii* (Ramírez & González) Roeijmans, van Eijk & Yarrow (1989)

Synonyms:
Candida huempii Ramírez & González (1984j)
Candida huempii Ramírez & González emend. Ramírez (1988)

Growth in 5% malt extract: After 3 days at 19°C, the cells are ovoidal to elongate, (2.7–6.7)×(8.0–16.1) μm, single or in pairs. After one month a slight sediment and ring are present.

Growth on 5% malt extract agar: After one month at 19°C, the streak culture is semi-glistening, cream-colored, butyrous and flat and the margin is entire.

Dalmau plate culture on corn meal agar: After one month at 19°C, primitive pseudomycelium is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	s
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	s	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	ws
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w
D-Arabinose	s	Inositol	–
D-Ribose	ws	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 20°C	+
50% (w/w) glucose–yeast extract agar	n	Growth at 30°C	–
10% NaCl/5% glucose	s		

Co-Q: Not determined.
Mol% G+C: Not determined.
Cell hydrolyzates: Glucose, mannose and xylose (Roeijmans et al. 1989).

Origin of the strain studied: A single strain was isolated from the advanced stage of decay of a fallen trunk of a laurel tree, *Laurelia sempervirens* Weim., in the evergreen rainy Valdivian forest of southern Chile near the Island of Chiloé in November–December 1980.

Type strain: CBS 8186 (Ramírez and González, strain 6049).

94.20. *Cryptococcus humicolus* (Daszewska) Golubev (1981)

Synonyms:
Torula humicola Daszewska (1912)
Mycotorula humicola (Daszewska) F.C. Harrison (1928)
Candida humicola (Daszewska) Diddens & Lodder (1942)
Azymoprocandida humicola (Daszewska) Novák & Zsolt (1961)
Apiotrichum humicola (Daszewska) von Arx & Weijman (1979)
Vanrija humicola (Daszewska) R.T. Moore (1980)
Candida suaveolens Langeron & Guerra 1938 [nec *Sachsia suaveolens* Lindner (1895); nec *Oospora suaveolens* (Lindner) Lindau (1907); nec *Oidium suaveolens* Krzemecki (1913); nec *Cylindrium suaveolens* (Krzemecki) Burns (1933)]
Sporobolomyces albidus Ramírez Gómez (1957)
Nadsonia slovaka Kocková-Kratochvílová & Svobodová-Poláková (1959)

Growth in 5% malt extract: After 3 days at 25°C, the cells are variable in form and size. They may be short-ovoidal, drop-like, lemon-shaped, long-ovoidal to cylindrical and measure (2.5–4.7)×(4.7–21.4) μm. After one month, a pellicle and sediment are present.

Growth on 5% malt extract agar: After one month

at 25°C, the streak culture is yellow, glistening to dull, smooth to densely rugoid.

Dalmau plate culture on corn meal agar: Pseudomycelium and true mycelium are abundantly formed. Hyphae and pseudohyphae are often wavy in appearance. Branching is characteristically at narrow angles resulting in branches that are almost parallel to the main axes, as seen through the bottom of the plate. The ovoidal blastoconidia may form laterally on small terminal hyphal projections. (Fig. 389).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	w/–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	s	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 9 (type strain); 9 (Yamada and Kondo 1972a).
Mol% G + C: 58.8–63.2, 3 strains (T_m: Nakase and Komagata 1971f).
Cell hydrolyzates: Glucose, mannose, xylose, galactose, glucuronic acid (von Arx and Weijman 1979).

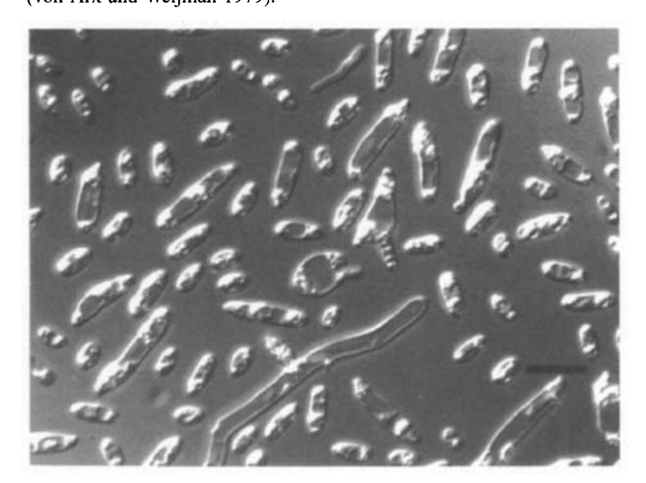


Fig. 389. *C. humicolus*, CBS 571. Cells grown on potato-dextrose agar at 25°C for 2 weeks. Bar = 10 μm.

Origin of the strain studied: Soil (1).

Type strain: CBS 571, isolated in Switzerland by Daszewska (1912) from heath soil.

Comments: In contrast to previous editions of "The Yeasts", *Apiotrichum porosum* is no longer considered a synonym of *C. humicolus*. The decision is based on LSU rRNA nucleotide sequence analyses (Fell et al. 1992) and the lack of yeastlike morphology of *A. porosum*. The hyphae lack blastospores and the colony has a fuzzy appearance due to the presence of tufts of aerial hyphae. Molecular sequence analysis of the LSU rRNA suggests that *C. humicola* is related to members of the genus *Trichosporon* (Guého et al. 1993).

Strains reported from other sources include: culture contaminant (1); mushrooms (2); decaying toadstools (2); skin (2); water (1). Nakase and Komagata (1971f) reported isolating strains from strawberries and moss.

94.21. *Cryptococcus hungaricus* (Zsolt) Phaff & Fell (1970)

Synonym:
Dioszegia hungarica Zsolt (1957)

Growth in 5% malt extract: After 3 days at 17°C, the cells are globose to ovoidal, (4.0–5.4) × (6.0–8.7) μm, and are single, in pairs or in small clusters; budding is multilateral and occasionally a bud is connected to the parent cell by a very short neck. A slight ring and a little sediment may be present. After one month there is a moderate brownish-red ring or islets and some sediment.

Growth on 5% malt agar: After one month at 20°C, the streak culture is reddish to slightly orange, semi-glossy, smooth, pasty with an entire margin.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	+
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+/w	Inositol	+
D-Ribose	+/w	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 20°C	+
10% NaCl/5% glucose	–	Growth at 30°C	–

Co-Q: 10 (Guého et al. 1993).
Mol% G+C: 56.5 (PC: Golubev and Vagabova 1977).
Cell hydrolyzates: Glucose, mannose, xylose (von Arx and Weijman 1979).

Origin of the strain studied: Soil in Tihany, Hungary (1).

Type strain: CBS 4214, isolated in 1946 by Zsolt from soil in Tihany, Hungary.

Comments: Strains were also isolated from flowers of *Lewesia pygmaea,* a portulaca (1), seawater (4) and leaves of cereals (1).

94.22. *Cryptococcus kuetzingii* Fell & Phaff (1967)

Growth in 5% malt extract: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2.7–6.0)×(4.0–10.0) µm, single, in pairs, short chains, or clusters. After one month, a slight ring and sediment may be present.

Growth on 5% malt agar: After one month at 20°C, the streak culture is cream to tan. The surface is smooth, glistening and the texture is so mucoid that the growth runs to the bottom of the slant.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	w
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	w	Inositol	+
D-Ribose	w	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Urease	+
Saccharate	w	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
10% NaCl/5% glucose	–	Growth at 37°C	–

Co-Q: Not determined.
Mol% G+C: 54.4 (T_m: Nakase and Komagata 1971c).
Cell hydrolyzates: Xylose, mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Medlar fruit (*Mespilus* sp.), isolated by G. Protii in Italy in 1953 (CBS 1926).

Type strain: CBS 1926.

Comments: Strains were also isolated from trachea rinsings of a patient by G.A. de Vries in the Netherlands, 1955 (CBS 2289); a strain of unknown origin (CBS 4234) contributed by G. Ricci (Rome, Italy); air in the Netherlands by A. Kikstra (CBS 6086) and dog's skin in the Netherlands by E.A.R.F. Baudet (CBS 922). After the completion of this manuscript, nucleotide sequence analysis in our laboratory (unpublished) indicates that *C. kuetzingii* is closely related to *C. albidus.*

94.23. *Cryptococcus laurentii* (Kufferath) C.E. Skinner (1950)

Synonyms:
Torula laurentii Kufferath (1920)
Torulopsis laurentii (Kufferath) Lodder (1934)
Torula aurea Saito (1922)
Chromotorula aurea (Saito) F.C. Harrison (1928)
Rhodotorula aurea (Saito) Lodder (1934)
Torula flavescens Saito (1922)
Torulopsis flavescens (Saito) Lodder (1934)
Cryptococcus flavescens (Saito) C.E. Skinner (1947b)
Cryptococcus laurentii (Kufferath) C.E. Skinner var. *flavescens* (Saito) Lodder & Kreger-van Rij (1952)
Rhodotorula laurentii (Kufferath) Hasegawa, Banno & Yamauchi (1960)
Torulopsis carnescens Verona & Luchetti (1936)
Rhodotorula peneaus Phaff, Mrak & Williams (1952)
Rhodotorula nitens Mackenzie & Auret (1963)

Growth in 5% malt extract: After 3 days at 25°C, the cells are spheroidal to ovoidal to elongate and are single, in pairs or in short chains of three or four cells. Cell sizes are (2.0–5.5)×(3.0–7.0) µm; some strains may have elongate cells which measure 8.5×13 µm. A thin ring, a moderate sediment and sometimes islets are present. After one month a moderate to thick slimy ring and a heavy sediment are present; a pellicle or islets may form.

Growth on 5% malt agar: After one month at 20°C, the streak culture is cream-colored, yellowish, pinkish, orange or tan colored. The surface is smooth and glossy, sometimes pasty (not mucoid); in most strains the texture is slimy so that the growth runs to the bottom of the slant. The margin is entire to lobate.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent or rudimentary.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+/w
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Yamada and Kondo 1973).
Mol% G+C: 51.2–59.0 (T_m: Nakase and Komagata 1971c); 58 (BD: Storck et al. 1969); 59.7 (BD: Baptist and Kurtzman 1976).
Cell hydrolyzates: Xylose, mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Palm wine isolated by Kufferath (1920) from "malafou" or palm wine from the Congo; wheat and corn isolated by Kurtzman (1973).

Type strain: CBS 139.

Comments: Strains were also isolated from other sources: Italian muscatel wine (1), bottle in brewery (1), slime flux of oak (1), tumor (1), bronchial tube of patient (1), leaves of tropical plants (4), insect frass in *Acacia karroo* (1), sea water (1), air (3), deep frozen beans (1), shrimp (1), soil (3) and beech-forest soil, Denmark (7).

Strains of opposite mating types from wheat and corn (CBS 6473, CBS 6474: MT-*α* and CBS 6475, CBS 6476: MT-*a*) were isolated and described by Kurtzman (1973). After conjugation, the zygote formed true mycelium with chlamydospore-like structures that lacked clamp connections. The complete life cycle was not determined. *C. laurentii,* as noted by strain differences in mol% G+C, is genetically heterogenous. This heterogeneity was confirmed by Guého et al. (1993) who reported differences in nucleotide sequence analysis of the mating strains and the type strain of *C. laurentii.*

94.24. *Cryptococcus luteolus* (Saito) C.E. Skinner (1950)

Synonyms:
Torula luteola Saito (1922)
Chromotorula luteola (Saito) F.C. Harrison (1928)
Torulopsis luteola (Saito) Lodder (1934)
Rhodotorula luteola (Saito) Hasegawa, Banno & Yamauchi (1960)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to elongate, (3.1–6.0)×(5.5–9.0) μm, and are single, in pairs or short chains; a ring begins to form and a light sediment is present. After one month a moderate amount of sediment and a ring are present. A thin film may also form.

Growth on 5% malt agar: After one month at 20°C, the streak culture is cream-colored to yellowish or tan to gray-brown. The surface is smooth and glossy, the texture slimy so that the growth runs to the bottom of the slant. The margin is entire.

Slide culture on potato agar: Pseudomycelium is absent or chains of cells are formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+/w
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	+/w	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	w	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 25°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Yamada and Kondo 1973).
Mol% G+C: 59.5 (T_m: Nakase and Komagata 1971c).
Cell hydrolyzates: Xylose, mannose low, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Air in Tokyo by K. Saito (1); foliage of tropical plants (2); coastal waters of the Bahamas by J. Fell (12); acidic sludge in the Netherlands by A.M. Jansen (1).

Type strain: CBS 943, from the air in Tokyo in 1922.

94.25. *Cryptococcus macerans* (Frederiksen) Phaff & Fell (1970)

Synonyms:
Rhodotorula macerans Frederiksen (1956)
Rhodotorula lini Wieringa (1956)

Cryptococcus hungaricus (Zsolt) Phaff & Fell var. *gallicus* Saëz (1973b)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal or long-ovoidal, single or in pairs, (3.0–4.5)×(5.0–15.0) µm. A thin ring and some sediment are present. After one month a moderate to heavy, slimy reddish ring and a heavy sediment are formed; islets may develop.

Growth on 5% malt agar: After one month at 20°C, the streak culture is bright coral-red to pink-peach, surface nearly smooth with some rugose or sectored areas, and glossy with entire margins. The texture is pasty.

Dalmau plate culture on corn meal agar: Pseudomycelium absent or rudimentary.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+/w	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+/w	Salicin	+/w
Inulin	–	D-Gluconate	s
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	+	Gelatin liquefaction	+
Saccharate	–	Biotin-free	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 25°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	–		
Starch formation	+		

Co-Q: 8 (Guého et al. 1993, Yamada and Kondo 1973).
Mol% G+C: 61.2 (PC: Golubev and Vagabova 1977).
Cell hydrolyzates: Xylose, mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Dew-retted flax, Denmark (3); pharynx of deer, *Axis axis* (2); flowers, Canada (2); spring water in California, USA (1), slime flux of elm (*Ulmus carpinifolia*) in California, USA.

Type strain: CBS 2206, isolated by Frederiksen (1956) from field-retted flax straw in Denmark. This species formed the major proportion of the red yeasts on this substrate, particularly during the winter months.

Comments: Mating types were reported by Rodrigues de Miranda (1984d); CBS 2206 and CBS 6532 are of one type (*a*), CBS 2425 and CBS 2426 of the opposite type (*α*). Rodrigues de Miranda did not describe a complete life cycle for this species; mating studies in our laboratory were unsuccessful. According to nucleotide sequence analysis (Guého et al. 1993), this species may be related to *Cystofilobasidium*; this relationship is also suggested by the presence of Co-Q 8, which is found in *Cystofilobasidium*.

94.26. *Cryptococcus magnus* (Lodder & Kreger-van Rij) Baptist & Kurtzman (1976)

Synonym:
Cryptococcus laurentii (Kufferath) C.E. Skinner var. *magnus* Lodder & Kreger-van Rij (1952)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to elongate, (3.5–15.0)×(4.5–45.0) µm, single, in pairs or in short chains of up to six cells. A ring begins to form and a little sediment is present. After one month a moderate ring and a fair amount of sediment are formed.

Growth on 5% malt agar: After one month at 19°C, the streak culture is cream-colored to tan. The surface is smooth, highly glossy and the texture slimy, so that the growth runs to the bottom of the slant. The margin is entire.

Slide culture on potato agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	w	Methanol	–
L-Sorbose	v	Ethanol	–
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	w
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	w	DL-Lactate	–
D-Xylose	w	Succinate	w
L-Arabinose	+	Citrate	w
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Yamada and Kondo 1973).
Mol% G+C: 49.0 (BD: Storck et al. 1969); 54.1 (BD: Baptist and Kurtzman 1976).
Cell hydrolyzates: Xylose, mannose low, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Atmosphere in the Netherlands.

Type strain: CBS 140, isolated by Sobels in 1943 from the atmosphere.

Comments: *C. laurentii* var. *magnus* was described by Lodder and Kreger-van Rij (1952) as distinct from *C. laurentii* var. *laurentii* on the basis of differences in cell morphology: the cells of var. *magnus* were found to be larger than those of var. *laurentii*, although Phaff and Fell (1970) could not separate them on this characteristic nor did we see the elongated cells. Baptist and Kurtzman (1976) raised the taxon to the rank of species based on differences in G+C content and comparative enzyme patterns. *C. magnus* can be separated from *C. laurentii* based on the inability of *C. magnus* to grow on melibiose and ribitol. Based on nucleotide sequence analysis (Yamada et al. 1990a), *C. magnus* may be related to *C. ater*, an observation that we have confirmed in our laboratory (unpublished).

94.27. *Cryptococcus marinus* (van Uden & Zobell) Golubev (1981)

Synonym:
Candida marina van Uden & Zobell (1962)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal to elongate, (2.7–6)×(5.4–12) µm, and may be single, in pairs or in chains. After one month, a dull pellicle and heavy sediment are present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is cream to brownish-yellow, dull, soft, smooth.

Dalmau plate culture on corn meal agar: The pseudomycelium may be primitive or consist of long wavy pseudohyphae bearing, globose and ovoidal, often curved cells that are often terminal or sometimes lateral on short pedicels (Fig. 390).

Fermentation: absent.

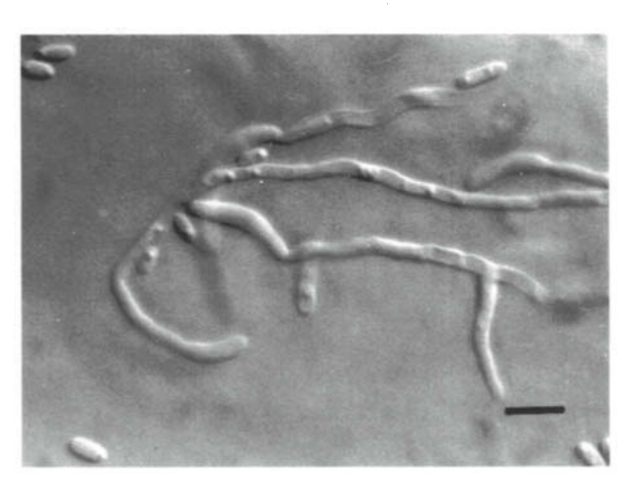


Fig. 390. *C. marinus*, CBS 5235. Cells grown on corn meal agar at 25°C for 2 weeks. Bar = 5 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	–
D-Arabinose	s	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Urease	+
5-Keto-D-gluconate	n	Gelatin liquefaction	–
Saccharate	–	Thiamine-free	–
D-Glucuronate	+	Pyridoxine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 25°C	+
10% NaCl/5% glucose	+	Growth at 30°C	–
Starch formation	–		

Co-Q: 10 (Yamada and Kondo 1972a).

Mol% G+C: 64.0 (T_m: Stenderup et al. 1972); 61.2 (T_m: Nakase and Komagata 1971c).

Cell hydrolyzates: Xylose, mannose low, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Seawater over coral and algal growth at the Great Barrier Reef, Torres Strait, Australia during July and August, 1960 (van Uden and Zobell 1962).

Type strain: CBS 5235.

Comments: Van Uden and Zobell (1962) did not indicate a type culture; van Uden and Buckley (1970) designated the strain listed above.

94.28. *Cryptococcus neoformans* (Sanfelice) Vuillemin (1901)

See *Filobasidiella neoformans* p. 656

94.29. *Cryptococcus podzolicus* (Bab'eva & Reshetova) Golubev (1981)

Synonym:
Candida podzolica Bab'eva & Reshetova (1975)

Growth in 5% malt extract: After 3 days at 25°C, the cells are polymorphic, including obpyriform, ovoidal, spheroidal to elongate, measuring (3.4–4.7)×(5.4–8.7) µm. They occur singly, in pairs and may bear lateral or polar elongate appendages. A light sediment is present. After one month a heavy sediment and a ring are present (Fig. 391).

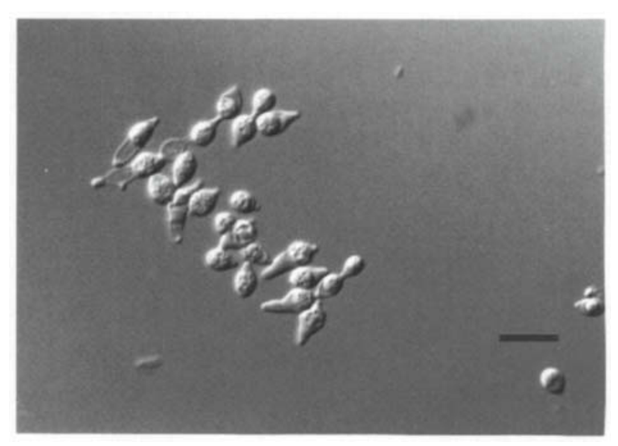


Fig. 391. *C. podzolicus,* CBS 6819. Cells grown on glucose–yeast extract water at 25°C for one week. Bar = 10 µm.

Growth on 5% malt extract agar: After one month at 25°C, the streak colony is white to cream or tan-colored, the surface is very rugoid, and the margin is fringed with true and pseudomycelium.

Dalmau plate culture on corn meal agar: True and pseudomycelium are present; buds are produced on short or elongate conidiophores.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	s
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	s
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	w
L-Arabinose	+	Citrate	s
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	+
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) glucose–yeast extract agar	–	Growth at 25°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G + C: 62.8 (Bab'eva and Reshetova 1975).

Extracellular polysaccharides: Glucose, galactose, mannose and xylose (Bab'eva and Reshetova 1975).

Origin of the strain studied: From a sod-podzolic soil in a birch forest (Chashnikovo, Solnechnogorsk district, Moscow Province, Russia).

Type strain: CBS 6819 (Bab'eva and Reshetova strain BKM Y-1982).

Comments: Bab'eva and Reshetova (1975) sampled the main soil and climatic zones of the European part of the Soviet Union, the Siberian North and the mountainous regions of the Pamirs, the Tien Shan, the Caucasus, the Crimea and the Altai (Lake Teletskoe region). They reported finding this species in podzolic and sod-podzolic soils in the taiga zone but not in the tundra. They also found it in bog and meadow (flood plain) soils of the same zone and farther south in the gray forest soils, as well as in red earth (Georgia) and brown forest soils in a pine forest in the Lake Teletskoe region. *C. podzolicus* was not found in black earth, nor chestnut soils, nor mountain soils of the Pamirs, the Tien Shan and the Crimea. They found the species in the soils and not on plants, from studies throughout the year. Sampaio and Fonseca (1995) reported several strains of *C. laurentii,* also isolated from soil, which are physiologically related to *C. podzolicus.*

We were unable to confirm the following assimilation test results from the original description: assimilation of sorbitol and salicin, negative, as well as starch production; assimilation of ribitol, positive and slow.

94.30. *Cryptococcus skinneri* Phaff & do Carmo-Sousa (1962)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to globose, (3.4–6.7) × (4.0–7.4) µm, single or in pairs. A light sediment is present after one month.

Growth on 5% malt agar: After one month at 25°C, the streak culture is white to cream-colored, smooth, glossy, slightly mucoid, raised and the margin is entire.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	v
Maltose	–	Erythritol	w/–
Cellobiose	s	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	ws
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	s	Citrate	+
D-Arabinose	s	Inositol	+
D-Ribose	v	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	Thiamine-free	–
D-Glucuronate	+	Growth at 25°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	w/–
		Growth at 37°C	–
10% NaCl/5% glucose	–		
Starch formation	+		

Co-Q: Not determined.

Mol% G+C: 53.0 (BD: Storck et al. 1969).

Cell hydrolyzates: Xylose, mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Insect frass underneath the bark of a young dying specimen of the Pacific coast hemlock (*Tsuga heterophylla*) located on the bank of the Siletz River in NW Oregon (Phaff and do Carmo-Sousa 1962).

Type strain: CBS 5029 (Phaff and do Carmo-Sousa strain UCD FST60-82).

Comments: Strains were also isolated from slime fluxes of willow (*Salix*) and aspen (*Populus*) spp. in Alaska, Yukon Territory and British Columbia.

94.31. *Cryptococcus terreus* di Menna (1954b)

Synonyms:

Cryptococcus himalayensis S. Goto & Sugiyama (1970)

Cryptococcus elinovii Golubev & Tauson (1979)

Growth in 5% malt extract: After 3 days at 25°C, the cells are mainly globose or short-ovoidal, single, in pairs or short chains, (3.5–6.5)×(4.0–8.0) µm. A light ring and a small amount of sediment may be present. After one month at 25°C, a moderate ring and a moderate to heavy sediment are formed. At 19°C in 2% glucose-peptone–yeast extract–water, a few cells were seen to bud on short stalks (Fig. 392).

Growth on 5% malt agar: After one month at 19°C, the streak culture is brownish cream-colored or yellowish-tan. The surface is smooth, glossy, and the texture is in some strains soft but not mucoid, in other strains the growth is so mucoid that it runs to the bottom of the slant. The margin is entire.

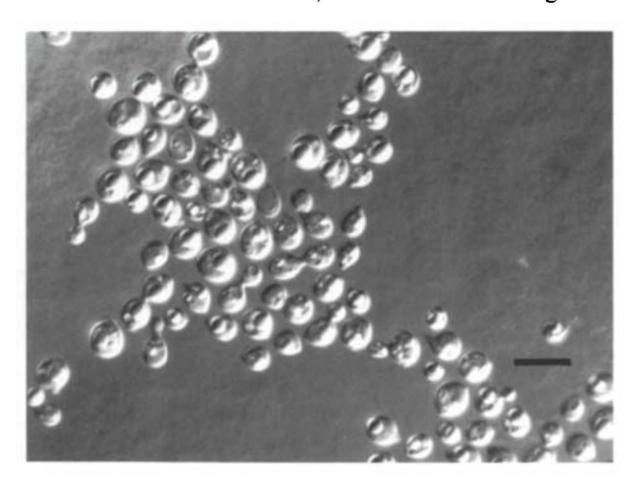


Fig. 392. *C. terreus*, CBS 1895. Cells with buds on short stalks grown in glucose–yeast extract water for one week at 19°C. Bar = 10 µm.

Dalmau plate culture on corn meal agar: Pseudomycelium absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	v
Sucrose	–	Glycerol	–
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	s	Galactitol	v
Lactose	s	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	w
L-Arabinose	+	Citrate	v
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	+
5-Keto-D-gluconate	+	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	w		

Co-Q: 10 (Yamada and Kondo 1973).

Mol% G+C: 59.5, *C. terreus* strain, CBS 1895 (BD: Storck et al. 1969); 54.7 (T_m: Vancanneyt et al. 1994); 57–58, *C. elinovii* strains (paper chromatography: Golubev and Tauson 1979); 55.4, 55.7 (paper chromatography: Golubev and Vagabova 1977, Golubev and Tauson 1979); 58.0, *C. himalayensis* strain (T_m: Baharaeen et al. 1982).

Cell hydrolyzates: Xylose, mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Soil in New Zealand by di Menna (18); type strain of *C. himalayensis,* CBS 6293, from soil sample from Chawa Gassar, Bhutan, May 1967, by H. Kanai; type strain of *C. elinovii,* CBS 7051, from soil on a high mountain in U.S.S.R. by Golubev.

Type strain: CBS 1895, isolated from soil in New Zealand, obtained from di Menna.

Comments: *Cryptococcus himalayensis* was described by Goto and Sugiyama (1970) as a separate species, because of the ability to assimilate sucrose, raffinose, and melezitose and the inability to assimilate maltose. Repeated tests by Rodrigues de Miranda (1984d) proved the opposite and this species fits the standard description for *Cryptococcus terreus.* A synonymous relationship between *C. elinovii* and *C. himalayensis* is evident when nuclear sequences are compared (Fell et al. 1992). The nuclear sequence alignment results also show a close relationship of *C. fuscescens* to *C. terreus.*

Strains were also isolated from desert soil in Chile by di Menna (3); soil in Belgian forest by L. Hennebert; soil in New York by S. Gochenaur, 1974; soil in northern California by Phaff (1); and from gut contents of trout caught in northern California by Spencer and Phaff (2).

94.32. *Cryptococcus uniguttulatus* (Zach) Phaff & Fell (1970)

See *Filobasidium uniguttulatum* p. 667

94.33. *Cryptococcus vishniacii* Vishniac & Hempfling (1979)

Synonyms:
Cryptococcus asgardensis Vishniac & Baharaeen (1982)
Cryptococcus baldrensis Vishniac & Baharaeen (1982)
Cryptococcus hempflingii Vishniac & Baharaeen (1982)
Cryptococcus lupi Baharaeen & Vishniac (1982)
Cryptococcus tyrolensis Vishniac & Baharaeen (1982)
Cryptococcus vishniacii Vishniac & Hempfling var. *asocialis* Vishniac & Baharaeen (1982)
Cryptococcus vishniacii Vishniac & Hempfling var. *vladimirii* Vishniac & Baharaeen (1982)
Cryptococcus vishniacii Vishniac & Hempfling var. *wolfii* Vishniac & Baharaeen (1982)
Cryptococcus wrightensis Vishniac & Baharaeen (1982)
Cryptococcus socialis Vishniac (1985a)

Growth in 5% malt extract: After 3 days at 19°C, the cells are spheroidal, ovoidal to elongate, (3.4–9.4)×(4.0–26) µm, and may be single, budding, or in chains.

Growth on 5% malt extract agar: After one month at 19°C, the colony is cream-colored, smooth, butyrous, semi-glistening with the edges entire.

Dalmau plate culture on corn meal agar: After one week at 19°C, pseudomycelium is not present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	v	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	–
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	s	Salicin	v
Inulin	v	D-Gluconate	v
Soluble starch	v	DL-Lactate	–
D-Xylose	v	Succinate	s
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	v	Nitrate	v
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Starch formation	+
5-Keto-D-gluconate	n	Urease	v
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 19°C	+
50% (w/w) glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: 9 (Vishniac and Baharaeen 1982).
Mol% G+C: 53.48–56.09 (T_m: Baharaeen and Vishniac 1982, Vishniac 1985a).
Cell hydrolyzates: Not determined.

Origin of the strains studied: Soils of the Dry Valleys, South Victoria Land, Antarctica, collected in 1973 by W.V. Vishniac and Z. Bowen: type strain of *C. asgardensis*, CBS 8141 (MYSW 302Y310); type strain of *C. baldrensis*, CBS 8142 (SW 302Y259); type strain of *C. hempflingii*, CBS 8143 (MYSW 306Y212); type strain of *C. lupi*, CBS 8100 (MYSW 202Y252); type strain of *C. tyrolensis*, CBS 8144 (MYSW 303Y336); type strain of *C. wrightensis*, CBS 8145 (MYSW 303Y206); type strain of *C. vishniacii* var. *asocialis*, CBS 8146 (MYSW 302Y312); type strain of *C. vishniacii* var. *vladimiri*, CBS 6810 (MYSW 302Y265); type strain of *C. vishniacii* var. *wolfii*, CBS 6812 (MYSW 303Y216).

Type strain: CBS 7110 (MYSW 304Y268), soil isolate (sandstone, sand) from Mt. Baldr, South Victoria Land, Antarctica.

Comments: According to the original description, optimal growth except for the type strain is 10°C, and 20°C for the other strains. We recommend 5°C for short term storage and cryopreservation for long term storage. The basis for the species listed in synonymy with *C. vishniacii* is similarity in nucleotide sequences (Fell et al. 1992, Guého et al. 1993) and results of standard taxonomic tests.

94.34. *Cryptococcus yarrowii* A. Fonseca & van Uden (1991)

Growth in 5% malt extract: After 3 days at 25°C, the cells are elongate cylindrical to fusiform, (1.1–2.7)×(3.2–7.4) µm, and reproduce predominantly by polar budding. They may be single, in pairs or in short chains. A slight sediment is formed. After one month, both a ring and sediment are present.

Growth on 5% malt agar: After one month at 25°C, the colony is white to cream-colored, slightly raised, semi-glistening, butyrous, and the margin is entire.

Dalmau plate culture on corn meal agar: Rudimentary pseudomycelium is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	s
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	s	Succinate	w
L-Arabinose	–	Citrate	w
D-Arabinose	s	Inositol	+
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	n	Gelatin liquefaction	–
Saccharate	w	Butanediol	–
D-Glucuronate	+	L-Malic acid	–
Arbutin	–	L-Tartaric acid	–
Xylitol	–	Sodium nitrate	+
L-Arabinitol	–	Glucono-δ-lactone	+
Cadaverine	+	D-Galacturonic acid	+
Creatinine	–	Propanediol	+
Creatine	–	Imidazole	–
L-Lysine	+/w	0.01% Cycloheximide	–
Ethylamine	+	0.10% Cycloheximide	–
50% (w/w) glucose–yeast extract agar	–	Growth at 25°C	+
10% NaCl/5% glucose	w	Growth at 30°C	–
Starch formation	–		

Co-Q: 10 (Fonseca and van Uden 1991).
Mol% G+C: 60.3 (T_m: Fonseca and van Uden 1991).
Cell hydrolyzates: Mannose, glucose, mannitol, rhamnose, galactose, xylose, inositol and arabitol.

Origin of the strain studied: From a decaying mushroom collected in a woods, Lisbon, Portugal, 1988.

Type strain: CBS 7417 (Fonseca and van Uden strain IGC 4525).

Comments: Sequence analysis of a partial region of the LSU rDNA in our laboratory indicates that *C. yarrowii* is a member of the genus *Rhodotorula* and that the cell wall analysis should be re-evaluated.

Comments on the genus

The generic name *Cryptococcus* was introduced by Kützing (1833) for an organism observed from dried scrapings collected from a window sill. Kützing's description was "Globuli museosi hyalini non colorati, in stratum determinatum mucosum facile secedens sine oidine aggregati". The organism was placed among the algae and a description of the only species, *C. mollis* Kützing, was not presented. In 1901, Vuillemin introduced the concept that *Cryptococcus* should be characterized by pathogenic yeasts and he transferred *Saccharomyces neoformans* Sanfelice 1895 to *Cryptococcus*. The history of the genus and the confusion caused by the two definitions of *Cryptococcus* can be found in Benham (1935), Lodder (1938), Skinner (1950) and Rodrigues de Miranda and Batenburg-van der Vegte (1981). In the meantime, nomenclature of the pathogen *Cryptococcus neoformans* was widely accepted by the medical community. Stability of that nomenclature was in question as taxonomic separations of *Torulopsis, Candida, Cryptococcus* and *Rhodotorula* came under scrutiny and von Arx and Weijman (1979) recommended the transfer of *C. neoformans* to *Apiotrichum*. In order to conserve the genus and retain stability of the binomial nomenclature of *C. neoformans*, the genus *Cryptococcus* Vuillemin was conserved with the type species *C. neoformans* (Fell et al. 1989, Greuter et al. 1994).

The first edition of "The Yeasts" (Lodder and Kreger-van Rij 1952) defined *Cryptococcus* as: cells surrounded by a capsule, formation of starch-like compounds, absence of pseudomycelium, inability to ferment and the lack of carotenoid pigments. Five species were accepted in the genus. The only change in the generic description through the 3rd edition of "The Yeasts" was the capacity of *Cryptococcus* species to assimilate inositol. *Cryptococcus* grew to 19 species. The major informative step, by this time, was the relationship of some species to the teleomorphic genera *Filobasidium* and *Filobasidiella*. The greatest pressure on *Cryptococcus* taxonomy was the retention of the ascomycetous species in *Candida* and the separation of basidiomycetous *Candida* species based on the composition of cell hydrolyzates (Weijman and Rodrigues de Miranda 1983). Species with xylose were incorporated into *Cryptococcus* and those species that lacked xylose were relegated to *Rhodotorula*. As a result, *Cryptococcus* expanded to 34 species.

The genus, as currently defined, is polyphyletic with tenuous boundaries based on a combination of abilities to utilize inositol and D-glucuronate and to synthesize starch (Table 74). This polyphyletic nature will have to be examined by a combination of molecular and classical systematics, particularly in consort with other members of the Filobasidiaceae and Tremellaceae. The goal should be a classification based on phylogenetic relationships and not the separation into several genera based on one or more artificial taxonomic characters. The latter type of taxonomy merely adds names and confusion to the literature and consequently hinders the ability to undertake the practical application of taxonomy viz., species identifications.

Separation of the 34 species assigned to *Cryptococcus* requires 2 morphological observations and 21 physiological tests, generally more time and effort than one wishes to devote to a routine identification. Therefore, we split all the species represented in Tables 74 and 75 into four groups (Tables 76–79) based on starch synthesis and assimilation of inositol, D-glucuronate and nitrate. Using these compounds as an initial screen, followed by testing the compounds in the appropriate Tables 76–79, it

is possible to reduce the number of tests required for a routine identification.

An alternative to classical taxonomy is the use of molecular identifications from nucleotide sequence analyses. Through use of species-specific oligonucleotide sequences as a primer in a polymerase chain reaction, a species can be rapidly identified. A primer for *C. neoformans* has been described (Fell 1995, Haynes et al. 1995) and primers for all species should be available in the near future.

A study of the molecular systematics of the genus is currently underway in our laboratory; some of the results are included under the comments of the individual species, which indicate relationships between certain species. We anticipate that, with the completion of this study, some changes within the genus will have to be made.

95. *Fellomyces* Y. Yamada & Banno

I. Banno and Y. Yamada

Diagnosis of the genus

Cells are spherical to ellipsoidal and produce one or more stalks, each giving rise to a single conidium. The conidium is freed without forceful ejection at the distal end of the stalk (Table 80). The stalks get longer than 4.0 μm. True mycelia may or may not be produced. Ballistoconidia are not formed.

Glucose is not fermented. Nitrate is not assimilated. Diazonium blue B color reaction is positive. Xylose is present in the cells. The coenzyme Q-10 system is present. D-Glucuronate and *myo*-inositol are assimilated.

Table 80
Salient characteristics differentiating the anamorphic stalked conidium-forming yeast genera *Fellomyces, Kurtzmanomyces, Sterigmatomyces* and *Tsuchiyaea*

Genus	Reproduction	CoQ system	Xylose in cells
Fellomyces	Conidium separated at distal end of long stalk	Q-10	+
Kurtzmanomyces	Conidium separated at distal end of long stalk, branching and elongating to form additional conidia	Q-10	–
Sterigmatomyces	Conidium disjointed in mid-region of short stalk	Q-9	–
Tsuchiyaea	Enteroblastic bud and conidium disjointed in mid-region of stalk	Q-9	+

Type species

Fellomyces polyborus (D.B. Scott & van der Walt) Y. Yamada & Banno

Species accepted

1. *Fellomyces fuzhouensis* (Yue) Y. Yamada & Banno (1988)
2. *Fellomyces horovitziae* Spaaij, Weber & Oberwinkler (1991)
3. *Fellomyces penicillatus* (Rodrigues de Miranda) Y. Yamada & Banno (1984)
4. *Fellomyces polyborus* (D.B. Scott & van der Walt) Y. Yamada & Banno (1984)

Key to species

See Table 81.

1. a Soluble starch and α-methyl-D-glucoside assimilated → 2
   b Soluble starch and α-methyl-D-glucoside not assimilated → 3

2(1). a True mycelium present .... *F. penicillatus*: p. 770
   b True mycelium absent .... *F. polyborus*: p. 771

3(1). a True mycelium present .... *F. fuzhouensis*: p. 769
   b True mycelium absent .... *F. horovitziae*: p. 769

Table 81
Key characters of species in the genus *Fellomyces*

Species	Assimilation		True mycelium	Mol% G+C
	Soluble starch	α-Methyl-D-glucoside		
Fellomyces fuzhouensis	–	–	+	55.2, 54.0
F. horovitziae	–	–	–	54.2
F. penicillatus	+	w	+	48.9
F. polyborus	+	s	–	49.6

Systematic discussion of the species

95.1. *Fellomyces fuzhouensis* (Yue) Y. Yamada & Banno (Yamada et al. 1988a)

Synonyms:
Sterigmatomyces fuzhouensis Yue (1982)
Sterigmatomyces tursiopsis Kurtzman, Smiley, Johnson & Hoffman (Fell et al. 1984a) nom. nud.

Growth in malt extract: After 3 days at 25–30°C, the cells are spheroidal, (2.4–6) μm, ovoidal, (2.37–5.5)×(2.8–6.2) μm, or sometimes irregularly shaped. Conidia are produced on stalks measuring 4–15 μm (Fig. 393). The number of stalks is 1–8 or more. The stalks may be sympodially formed. The conidia may produce additional stalks, and conidia may form in branched chains of cells. The conidia are separated at the distal end of the stalks from parent cells. The conidium is pear-shaped or bottle-shaped. True mycelium may be produced. A ring, powdery pellicle and sediment are present.

Growth on malt agar: After 3 days at 25°C, the streak culture is cream-colored, pasty, with a dry, dull and wrinkled surface. The margin is crenulate. True mycelium and filamentous elongation of the cells occur. A variant frequently occurs, producing a smooth, glistening colony with an entire margin. The conidia may be produced on short stalks that give the appearance of budding cells.

Dalmau plate culture on corn meal agar: True mycelium (<2 μm wide) is branched with lateral and terminal stalks, rarely septate. Terminal conidia may develop from numerous stalks, resembling a penicillate head. Single or clustered blastoconidia may be produced on the hyphae.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	v	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	–
Melezitose	w/–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

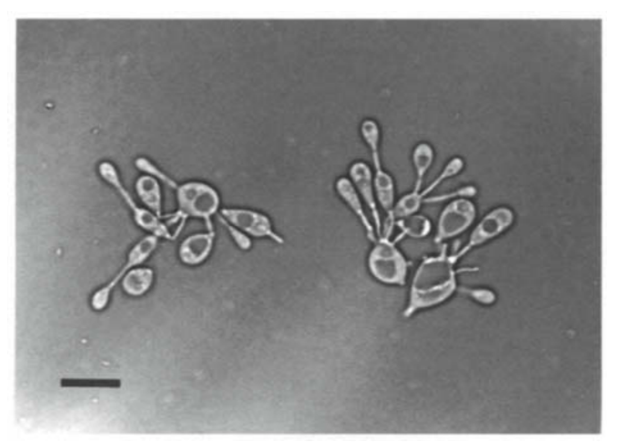


Fig. 393. *F. fuzhouensis*, IFO 10374. After 48 hours at 25°C in malt extract. Bar = 10 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	w
5-Keto-D-gluconate	+	Starch formation	v
Ethylamine	–	Urease	w/+
Arbutin	+	Growth at 37°C	–
50% Glucose	–		

Co-Q: 10 (Yamada and Banno 1984a).
Mol% G+C: 54.0 (HPLC), type strain IFO 10374; 55.2 (T_m), CBS 6133.
Xylose in cells: Present.

Origin of the species: IFO 10374 (CBS 8243; NRRL Y-12916; 1519C) flower of mango, J.-Z. Yue, China; IFO 10205 (CBS 6133; NRRL Y-7956), type strain of *Sterigmatomyces tursiopsis* nom. nud., bottlenosed dolphin (*Tursiops truncatus*), D.G. Ahearn, USA.
Type strain: CBS 8243 (IFO 10374).

95.2. *Fellomyces horovitziae* Spaaij, Weber & Oberwinkler (1991)

Growth in 5% malt extract: After 5 days at 25°C, the cells are globose to ovoidal, (3.0–8.0)×(2.0–6.0) μm, reproduce by enteroblastic budding or by forming conidia on stalks. A sediment, ring, pellicle and islets are formed. After 4 weeks at 25°C, a sediment, ring and a creeping pellicle are present.

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are globose, ellipsoidal to ovoidal, occasionally asymmetric, (2.5×6.0)×(2.0–6.0) μm. They occur singly or in pairs connected by stalks. They reproduce by enteroblastic budding or by forming conidia on stalks. The conidium detaches by disjunction at a septum in the distal region of the stalks. The mature conidia are clavate with a truncate base. They measure (2.0–4.0)×(1.5–2.5) μm. Parent cells are as a rule lipid-rich, forming 1–8 stalks. The size of the stalks is variable. The streak culture is mucoid, creamish-white with an entire margin. The culture surface is glistening with dull areas, caused by the formation of conidia on stalks.

Dalmau plate culture on corn meal agar: Neither hyphae nor pseudohyphae are formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	–
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	s
L-Arabinose	s	Citrate	s
D-Arabinose	s	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	50% (w/w) Glucose–yeast extract agar	–
5-Keto-D-gluconate	–		
Saccharate	n	10% NaCl/5% glucose	n
D-Glucuronate	+	Starch formation	–
Xylitol	s	Urease	+
L-Arabinitol	–	Nitrite	–
Arbutin	+	Gelatin liquefaction	–
Cadaverine	–	Glucono-δ-lactone	+
Creatine	–	0.1% Cycloheximide	+
Creatinine	–	Growth at 25°C	+
Ethylamine hydrochloride	s	Growth at 37°C	–
L-Lysine	s		

Co-Q: 10 (Spaaij et al. 1991).
Mol% G+C: 54.2 (T_m: Spaaij et al. 1991).
Xylose in cells: Present (Spaaij et al. 1991).

Origin of the strain studied: Fruit-body of *Xenasmatella* sp. collected in May 1981, at Hagelloch (Hornkopf) near Tübingen, Germany.

Type strain: CBS 7515 (Spaaij's strain FS-H-0043).

Comments: This species was not part of the original manuscript as received from Banno and Yamada; consequently the description was included in this chapter by the editors. The description presented above was extracted from Spaaij et al. (1991). Data supplied by CBS differed as follows: soluble starch, +; L-arabinose, +; L-rhamnose, +; ribitol, s; α-methyl-D-glucoside, s; inositol, s; arbutin D, –; growth at 25°C, –. CBS reported a second strain, CBS 7727, from rotting wood, Uppsala Stadsskogen, Sweden, that was isolated in May 1985 by O. Constantinescu.

Spaaij et al. (1991) distinguished *Fellomyces horovitziae* from *F. fuzhouensis*, *F. penicillatus* and *F. polyborus* by physiological differences in the utilization of L-rhamnose, erythritol, ribitol, mannitol, galactitol, *myo*-inositol and D-gluconate. An additional diagnostic character is the formation of true hyphae by *F. penicillatus* and *F. fuzhouensis* in contrast to an absence of hyphae or pseudohyphae in *F. horovitziae.*

95.3. *Fellomyces penicillatus* (Rodrigues de Miranda) Y. Yamada & Banno (1984a)

Synonym:
Sterigmatomyces penicillatus Rodrigues de Miranda (1975)

Growth in malt extract: After 3 days at 24°C, the cells, (1–6)×(2–11) μm, have a variety of shapes that may be spheroidal, ovoidal, obpyriform and apiculate and propagate by formation of blastic cells (conidia) at the top of stalks (Fig. 394). When attached to short hyphal strands, the cells are ventricose. The conidiogenous stalks (1–6 per cell) range in size from 0.5×(0.7–9) μm. After 30 days the majority of the cells are (1–7)×(2–8) μm with as many as 17 stalks per cell. The stalks range in size from 0.5×(0.7–35) μm. Conidium separation is at the distal position where an adjacent septum is formed.

Growth on malt agar: After 3 days at 24°C, the cell sizes are similar to those in malt extract and the stalks (1–15 or more per cell) are simple or branched, with a length of 40 μm or greater. The streak culture is dull, white to pale luteus, pasty, soft, and rugose with an irregularly crenulate border. Variation of colonies is frequently found which are smooth, glistening, soft, mucoid, raised, pale luteus to cream-colored with a border that is entire. The cells in these colonies are spheroidal to ovoidal, (2–3)×(3–4) μm. Stalks are few in the smooth colony type, thus the cells have the appearance of typical budding yeast cells.

Dalmau plate culture on corn meal agar: Rugose colonies develop branched true mycelium (<2 μm wide); cross walls are rare, stalks are lateral, short to elongated, with terminal conidia. The latter may develop numerous stalks with conidia to give the appearance of a penicillate head. Hyphae may also develop in the smooth colonies, however, the majority of the cells are similar to those on malt agar.

Fermentation: absent.

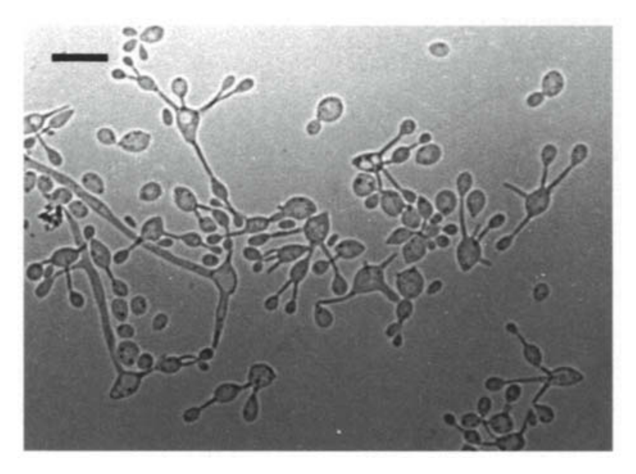


Fig. 394. *F. penicillatus*, IFO 10119. After 72 hours at 25°C in malt extract. Bar = 10 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	w
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	s
Lactose	+	D-Mannitol	+
Melibiose	w	D-Glucitol	+
Raffinose	w	α-Methyl-D-glucoside	w
Melezitose	+	Salicin	w
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	n
5-Keto-D-gluconate	+	Starch formation	w
Arbutin	w	Urease	+
Ethylamine	–	Growth at 37°C	+
50% Glucose	–		

Co-Q: 10 (Yamada and Banno 1984a).
Mol% G + C: 48.9 (T_m), type strain (CBS 5492).
Xylose in cells: Present.

Origin of the strains studied: IFO 10119 (CBS 5492), contaminant on agar plate, G. Kraepelin, Germany; CBS 7046, birch tree, I.P. Bab'eva, U.S.S.R.; CBS 7152, woodland soil, J.P. van der Walt, South Africa.

Type strain: CBS 5492 (IFO 10119).

Comments: *Sterigmatomyces penicillatus* was considered to be a later, facultative synonym of *S. polyborus* by Jong and King (1977a), since the type strains of the two species were morphologically and physiologically similar. In contrast, Kurtzman (in Fell et al. 1984a) and Kurtzman (1990a) confirmed by DNA reassociation that *S. penicillatus* and *S. polyborus* are distinct. Yamada et al. (1986a,b) reported that the two species are separable at the specific level by electrophoretic patterns of seven enzymes. In this monograph, therefore, the authors regard *F. penicillatus* as a separate species on the basis of the low homology of DNA reassociation and of the different electrophoretic enzyme patterns from *F. polyborus*. The latter is distinguished morphologically from the former by absence of true mycelium.

95.4. *Fellomyces polyborus* (D.B. Scott & van der Walt) Y. Yamada & Banno (1984a)

Synonym:
Sterigmatomyces polyborus D.B. Scott & van der Walt (1970b)

Growth in malt extract: After 3 days at 24°C, the cells are spheroidal to ovoidal, (1.3–4.0)×(2.5–5.5) µm. The cells may be single or reproduce by the formation of single conidia on stalks that range in size from 0.5×(0.7–10) µm (Fig. 395). After one month there may be as many as 15 stalks per cell. Newly formed cells generally separate at the distal end of stalks. Islets, a light ring and sediment are present.

Growth on malt agar: After 3 days at 24°C, the streak culture is restricted, dull, creamy-white, powdery, and rugose with irregularly crenulate margins. The cells are similar to those in malt extract. Variants are present that form colonies which are glistening, raised, smooth and pale luteus. The margin is entire. The encapsulated cells bear few short (<0.5 µm in length) stalks. The cells appear similar to budding cells of the genus *Cryptococcus*.

Dalman plate culture on corn meal agar: Non-septate, hypha-like elongation of the cells occurs. Neither pseudomycelium nor true mycelium is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	s	D-Mannitol	+
Melibiose	s	D-Glucitol	+
Raffinose	v	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	ws
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

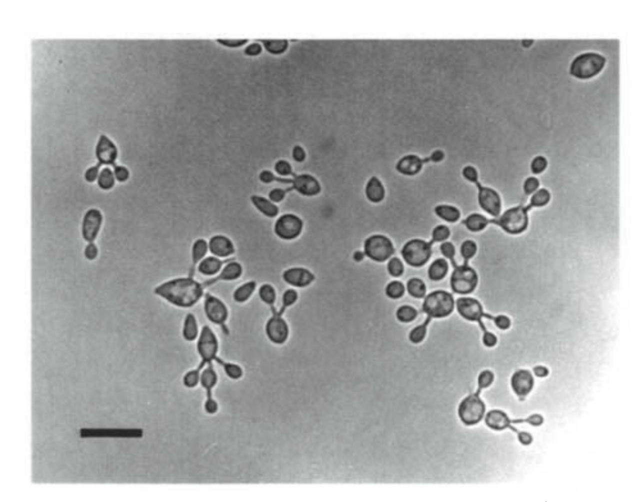


Fig. 395. *F. polyborus*, IFO 10203. After 48 hours at 25°C in malt extract. Bar = 10 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	10% NaCl/5% glucose	+
5-Keto-D-gluconate	+	Starch formation	−
Arbutin	s	Urease	+
Ethylamine	−	Growth at 35°C	−
50% Glucose	−		

Co-Q: 10 (Yamada and Banno 1984a).
Mol% G+C: 49.6 (T_m), type strain (CBS 6072).
Xylose in cells: Present.

Origin of the strains studied: IFO 10120 (CBS 6072), tunnels of *Xyleborus torquatus* in *Cussonia umbellifera* tree, J.P. van der Walt, South Africa; IFO 10203 (CBS 6643), ambergris, J. Nicot.

Type strain: CBS 6072 (IFO 10120).

Comments on the genus

Yeasts of the genus propagate mainly by conidiogenesis. A conidiogenous cell develops slender projections, on which a conidium is vegetatively produced. The conidia are never forcibly discharged. Fell (1966) and Fell et al. (1984a) called the projection bearing the conidium, "sterigma". The term "sterigma" should be strictly used for the ballistospore-bearing projection according to Snell and Dick (1957). Here, the term "stalk" is used for the projection. Nakase et al. (1989b) applied the term "non-ballistosporous conidium" to a conidium produced on the stalk. However, "non-ballistosporous conidium" does not specify this type of conidium as all blastoconidia are implied by this term. In this monograph, consequently the term "stalked conidium" is used for the conidium produced on a stalk and disjointed at the mid-region or at the distal position of the stalk according to van der Walt et al. (1987d) and Nakase et al. (1991b).

The stalked conidium-forming yeast genera *Fellomyces, Kurtzmanomyces, Sterigmatomyces* and *Tsuchiyaea* are phylogenetically separate from each other at the generic level on the basis of the comparison of the partial sequences of 18S and 26S (or 25S) ribosomal RNAs (Yamada et al. 1989b, Guého et al. 1990). The genus *Sterigmatosporidium* appears not to be a perfect state of the genus *Fellomyces,* although the two genera share the following characteristics: the formation of conidia which are freed by an end-break in the stalks, coenzyme Q-10 and presence of xylose in the cells (Kraepelin and Schulze 1982, Fell et al. 1984a, Yamada et al. 1986a, 1988a).

96. *Hyalodendron* Diddens

G.S. de Hoog and M.Th. Smith

Diagnosis of the genus

Colonies are dry, farinose to velvety, and white to cream-colored. Budding cells and pseudomycelium are absent or rare. Hyphae are hyaline, coherent or locally disarticulating. Conidia arise in short chains and are ellipsoidal to cylindroidal with truncate ends.

Sugars are not fermented. Nitrate is not assimilated. *myo*-Inositol is assimilated. Urease reaction is positive. Diazonium blue B reaction is positive. Dolipores are present. Coenzyme Q-9 system is present. Xylose is present in cell walls.

Type species

Hyalodendron lignicola Diddens

Species accepted

1. *Hyalodendron lignicola* Diddens (1934)

Systematic discussion of the species

96.1. *Hyalodendron lignicola* Diddens (1934)

Synonyms:
Hyalodendron lignicola Diddens var. *undulatum* Diddens (1934)
Hyalodendron lignicola Diddens var. *simplex* Diddens (1934)

Growth on potato carrot agar: After 10 days at 20–22°C, colonies are dry, flat, farinose, white, and vaguely zonate. True hyphae are thin-walled, often flexuose, 1.5–2.5 μm wide, and profusely branched at acute angles. Conidia arise in lateral or terminal, mostly unbranched chains comprising up to 10 conidia; conidia are ellipsoidal with truncate ends or cylindroidal with slightly swollen median parts and measure (1.5–3.0)×(5–15) μm (Fig. 396). Pale olivaceous, firm-walled inflated cells up to 30 μm in diameter are occasionally present in old cultures.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+/w
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	n
Soluble starch	v	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	w/–
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	n	Vitamin-free	w

Additional assimilation tests and other growth characteristics:

Urease	+	Growth at 37°C	–

Co-Q: 9, CBS 220.34 (Guého et al. 1993).
Mol% G+C: Not determined.

Origin of the strains studied: CBS 219.34, 220.34, paratype strains of *Hyalodendron lignicola,* from

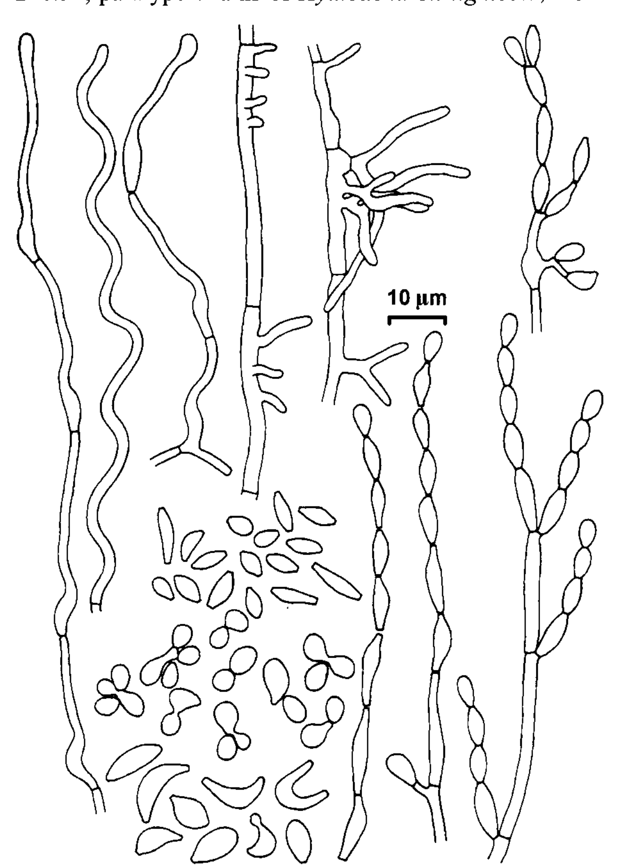


Fig. 396. *H. lignicola*, various strains. Hyphae with chains of blastoconidia, liberated conidia and budding cells.

woodpulp, Sweden, Melin; CBS 221.34, type strain of *Hyalodendron lignicola* var. *simplex,* woodpulp, Sweden, Melin; CBS 222.34, type strain of *Hyalodendron lignicola* var. *undulatum,* from wood pulp, Sweden, Melin; from beer pipes at brewery (1).

Type strain: CBS 219.34.

Comments on the genus

The species sporulates well on nutritionally poor media. The rather coherent chains of conidia with truncate ends are characteristic.

The single species in this genus has long been regarded as a hyphomycete since it forms dry colonies with true hyphae and mostly lacks budding cells. However, Martínez (1979) demonstrated a basidiomycetous nature on the basis of cell wall ultrastructure. The dolipore structure is identical to that known in the genus *Trichosporon* (Guého et al. 1992b). From partial 26S rRNA sequences, the species was found among other *Trichosporon* species (Guého et al. 1993). Also, the lack of fermentation and assimilation of many carbon compounds, indicates a close affinity to *Trichosporon.* The cell walls contain xylose (Weijman 1979a) which further indicates a tremellaceous relationship.

No teleomorph is known. De Hoog (1979a) described the occurrence of large, chlamydospore-like cells, of which the karyology has not yet been elucidated.

97. *Itersonilia* Derx

T. Boekhout

Diagnosis of the genus

Mycelium is hyaline, septate, and dikaryotic, or more rarely monokaryotic. Clamp connections are present in dikaryophase, but incomplete clamps occur in hyphal monokaryophase. Septa have dolipores without parenthesomes. Inflated cells ("sporogenous cells") are globose, subglobose, pyriform or ovoidal, and single or in clusters, terminal or intercalary, and thin to somewhat thick-walled. Ballistoconidia are bilaterally symmetrical, lunate or, more rarely, subpyriform, and formed either by germination of inflated cells or as secondary ballistoconidia. Germinating ballistoconidia give rise to appressoria. Chlamydospores are sometimes present. Monokaryophase is mostly yeastlike.

Sugars are not fermented. Diazonium blue B and urease reactions are positive. Xylose is present in whole-cell hydrolyzates. The major ubiquinone is Q-9.

Type species

Itersonilia perplexans Derx

Species accepted

1. *Itersonilia perplexans* Derx (1948)

Systematic discussion of the species

97.1. *Itersonilia perplexans* Derx (1948)

Synonyms:
Itersonilia pyriformans Nyland (1949)
Itersonilia pastinacae Channon (1963)

Description of the hyphal phase on yeast–peptone–glucose agar: After 5 days at 20°C, colonies are flat or somewhat raised, dull, cream, arachnoid to velutinous, with aerial fascicles, and with the margin entire or eroded. Hyphae are regularly branched, with cells of (50–120)×(2.0–7.0) µm. Inflated cells ("sporogenous cells") are intercalary or terminal on hyphae or hyphal branchlets, single or in clusters (10.0–18.0)×(8.0–15.0) µm, and germinating with hyphae or sterigmata (Fig. 397A). Ballistoconidia are bilaterally symmetrical, lunate to subpyriform (10.0–22.0)×(6.0–14.0) µm, and germinate with secondary ballistoconidia or appressoria. Appressoria are stalked or not, ellipsoid, circular or elongate in outline, and usually with dichotomously lobed margin (Fig. 397B). Chlamydospores are sometimes present and are subglobose (13.0–20.0)×(10.0–14.0) µm.

Growth of yeast phase on 5% malt extract agar: After 7 days at 17°C, the cells are ellipsoidal, allantoid, cylindrical or fusiform (9.0–40)×(3.0–11.0) µm, and single (Fig. 398A). Budding is usually polar, sometimes lateral, sessile or on short denticles, and with sympodial or percurrent proliferation. Occasionally, cells grow with short hyphae or revert to monokaryotic hyphae with incomplete clamp connections and may have inflated cells and chlamydospores. Colonies are butyrous or rather dry, smooth, transversely ridged or reticulate, shiny or dull, pale yellowish-brown, and with the margin entire, straight or crenulate.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on corn meal agar: After 7 days at 17°C, pseudohyphae may be present (Fig. 398B). Yeast phases sometimes revert to monokaryotic hyphae, which form incomplete clamp connections, inflated cells, ballistoconidia and chlamydospores. Aerobic growth is cream to pale yellowish-brown, dull or shiny, butyrous or rather dry, flat or slightly raised, smooth, transversely ridged or reticulate, with the margin entire, straight or crenulate. The colonies of reverted monokaryotic hyphal strains are whitish to cream, velutinous to hirsute, and morphologically indistinguishable from those of the dikaryotic phase.

Formation of ballistoconidia: Ballistoconidia are formed abundantly by both the di- and monokaryotic hyphal phases, are bilaterally symmetrical, lunate, ovoidal or subpyriform, and measure (10.0–22.0)×(6.0–13.5) µm.

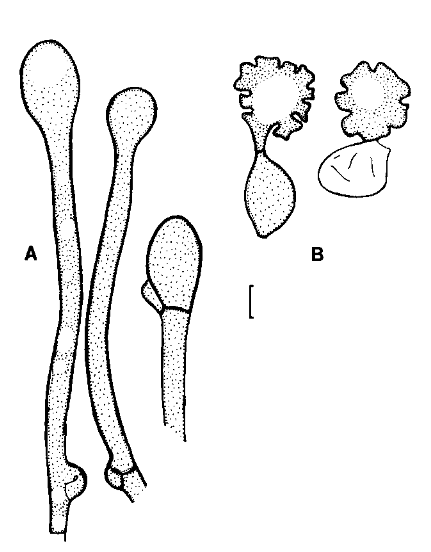


Fig. 397. *I. perplexans*, CBS 363.85. (A) Dikaryotic hyphae with inflated cells. (B) Appressoria. Bar = 5 µm.

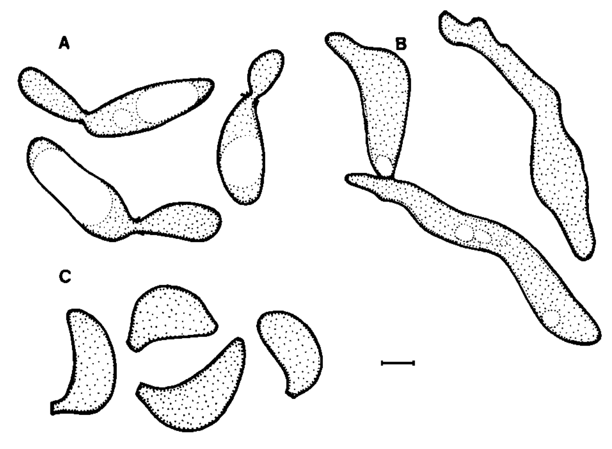


Fig. 398. *I. perplexans*, CBS 363.85. (A), (B) Yeast cells on MEA and morphology agar, respectively. (C) Ballistospores of the dikaryophase (MEA). Bar = 5 μm.

Bilaterally symmetrical ballistoconidia are occasionally formed by yeast cells (Fig. 398).

Fermentation: absent.

Assimilation (at 17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	v	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	v
Inulin	v	D-Gluconate	v
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	v
D-Ribose	v	Hexadecane	n
L-Rhamnose	v	Nitrate	v
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	v
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 9, CBS 144.68, 286.50, 197.53, 356.64 (Yamada and Konda 1984).

Mol% G + C: 60.1–63.9, eleven strains (T_m: Boekhout et al. 1991b).

Origin of the strains studied: CBS 197.53Y, yeast phase isolated from CBS 197.53, parsnip (*Pastinaca sativa*), G. Sowell, U.S.A; CBS 144.68Y, yeast phase isolated from CBS 144.68, air, A. Kikstra, Netherlands; CBS 363.85, wood of beech (*Fagus sylvatica*), C.T. Ingold, Great Britain, and CBS 363.85Y, yeast phase isolated from CBS 363.85; PD 85/1013Y, yeast phase isolated from PD 85/1013, curly kale (*Brassica oleracea*), W. Loerakker, Netherlands.

Complementary mating types: Four *A* and two *B* factors have been identified and were arbitrarily assigned as follows: A_1B_1 (CBS 363.85Y, CBS 144.68Y, PD 85/1013Y), A_1B_2 (CBS 197.53Y and PD 87/693Y), A_2B_2 (isolate "Boekhout"Y), A_3B_2 (PD 85/1007Y) and A_4B_2 (CBS 286.50Y) (Boekhout 1991b).

Type strain: CBS 363.85 (neotype, designated by Boekhout et al. 1991b).

Comments on the genus

Itersonilia perplexans is characterized by the presence of hyphae with clamp connections, inflated cells ("sporogenous cells"), and ballistoconidia. No teliospores occur. The species inhabits the phyllosphere, and dikaryotic strains of *Itersonilia* are reported to be pathogenic to parsnip (*Pastinaca sativa*) and chrysanthemums (*Chrysanthemum* sp.) (Channon 1956, 1963, Gandy 1966, Boekhout 1991b, Boekhout et al. 1991b). Isolates from parsnip were described as *I. pastinacae* (Channon 1963). However, "interspecific" matings, rather high DNA similarities and an intergrading morphology were observed (Boekhout 1991b, Boekhout et al. 1991b).

In mating experiments yeast strains were mixed thoroughly on Flegel's conjugation medium (Flegel 1976), which was slightly modified as suggested by van der Walt (see Boekhout 1991b). Two or three streaks of the mixed strains were made on the agar surface. All mating experiments were performed at 17°C.

Yeast phases may be formed under different environmental conditions, e.g., high humidity or submerged growth. Yeast cells of *I. perplexans* have never been isolated from nature. Nitrate-assimilating and non-assimilating yeast strains were observed (Boekhout 1991b). *Itersonilia* seems to have a tetrapolar mating system, and the life cycle comprises dikaryotic hyphal phases, and monokaryotic yeast- and hyphal phases. No karyogamy has been observed (Olive 1952, Boekhout 1991b).

Tremellales affinity is supported by the presence of dolipores without parenthesomes (Kreger-van Rij and Veenhuis 1971a, Boekhout 1991b), the presence of xylose in whole-cell hydrolyzates (Boekhout 1987, Weijman and Golubev 1987), 5S rRNA nucleotide sequences (Gottschalk and Blanz 1985), and sensitivity to killer toxins of *Cryptococcus laurentii* (Golubev 1990a, Golubev and Kuznetsova 1989).

98. *Kockovaella* Nakase, Banno & Y. Yamada

T. Nakase and I. Banno

Diagnosis of the genus

Vegetative cells are spherical, short ovoidal, ovoidal or kidney-shaped, and reproduce by stalked conidia and ballistoconidia or, rarely, by budding cells. Ballistoconidia are globose, napiform, kidney-shaped or ellipsoidal.

Fermentation is absent. Diazonium blue B color test is positive. The major ubiquinone is Q-10. Xylose is present in the cells.

The teleomorph is unknown.

Type species

Kockovaella thailandica Nakase, Banno & Y. Yamada

Species accepted

1. *Kockovaella imperatae* Nakase, Banno & Y. Yamada (1991)
2. *Kockovaella thailandica* Nakase, Banno & Y. Yamada (1991)

Key to species

See Table 82.

1. a Ballistoconidia kidney-shaped or ellipsoidal; galactitol assimilated .... *K. thailandica*: p. 778
   b Ballistoconidia globose or napiform; galactitol not assimilated .... *K. imperatae*: p. 777

Table 82
Key characters of species in the genus *Kockovaella*

Species	Assimilation of galactitol	Growth at 31°C	Mol% G+C		Ballistoconidia
			T_m	HPLC	
Kockovaella imperatae	+	–	52.3	49.0	globose, napiform
K. thailandica	–	+	49.5	47.7–48.4	kidney shaped, ellipsoidal

Systematic discussion of the species

98.1. *Kockovaella imperatae* Nakase, Banno & Y. Yamada (Nakase et al. 1991b)

Growth in YM broth: After 3 days at 25°C, the cells are spherical to ovoidal, (1.5–5.9)×(4.0–10.0) µm. They propagate by conidiation on a sterigma-like stalk, and by budding. The conidia are produced holoblastically on a stalk which develops multilaterally and holoblastically from the parent cell. Occasionally chains or clusters of conidia are formed. Stalks measure 0.3–0.6 µm in width and 4–10 µm in length. Newly formed cells generally separate at the distal position of the stalk. A sediment is formed. After one month at 17°C, a fragile, incomplete pellicle and a sediment are present. There may be as many as 5 stalks on a cell.

Growth on YM agar: After one month at 17°C, the streak culture is grayish-yellow to grayish-orange, delicately wrinkled, dull, soft to butyrous and an entire margin. Stalked conidia and ballistoconidia are produced.

Dalmau plate culture on corn meal agar: Mycelium and pseudomycelium are not formed.

Production of ballistoconidia: Ballistoconidia are produced on corn meal agar and YM agar. They are globose or napiform, (1.4–4.6)×(2.1–7.4) µm, and produced on sterigmata, which are generally 4.0–5.2 µm in length, but occasionally become much longer than 30 µm. Sterigma may be branched with a terminal ballistoconidium (Fig. 399).

Fermentation: absent.

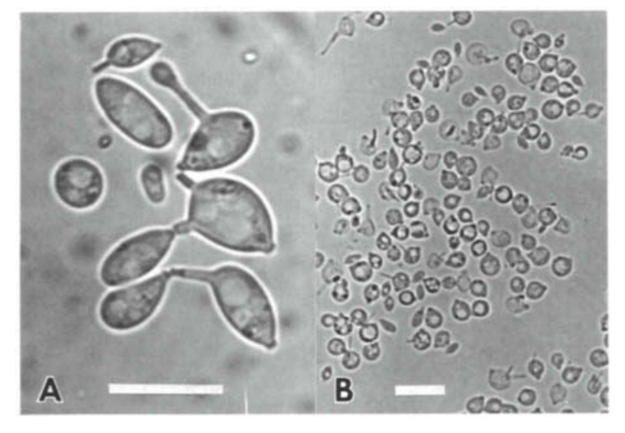


Fig. 399. *K. imperatae*, CBS 7554. (A) Vegetative reproduction by conidiation on stalks produced by parent cells after 3 days at 24°C on YM agar. (B) Ballistoconidia ejected from a colony on corn meal agar after 5 days at 22°C. Some ballistoconidia produce secondary ballistoconidia. Bars = 10 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	w/–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	w/–
Trehalose	+/l	Galactitol	+/l
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+/w
Raffinose	+/l	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	+/l
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+/w
L-Arabinose	+	Citrate	w/–
D-Arabinose	+/l	Inositol	w
D-Ribose	+	Hexadecane	–
L-Rhamnose	+/l	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+/w	Urease	+
5-Keto-D-gluconate	w	Gelatin liquefaction	w
D-Glucuronate	+	Acid production	–
D-Galacturonate	+	Thiamine-free	–
Starch formation	–	Growth at 30°C	–

Co-Q: 10 (Nakase et al. 1991b).
Mol% G+C: 52.3 (T_m); 49.0 (HPLC).
Xylose in cells: Present.

Origin of the strain studied: CBS 7554 (JCM 7826), a dead leaf of blady-grass (*Imperata cylindrica*), Nakase and Fungsin, Thailand.

Type strain: CBS 7554 (JCM 7826).

Comments: *K. imperatae* resembles *K. thailandica* in biochemical and physiological characteristics but can be separated by ballistoconidia morphology, which is symmetrical in the former and asymmetrical in the latter. DNA–DNA reassociation experiments clearly indicate the presence of two species (Nakase et al. 1991b).

98.2. *Kockovaella thailandica* Nakase, Banno & Y. Yamada (Nakase et al. 1991b)

Growth in YM broth: After 3 days at 25°C, the vegetative cells are spherical to ovoidal, (1.6–4.6)×(2.0–7.7) µm, single, in pairs, or in clusters, and reproduce by conidiation or rarely by budding. Conidia are produced holoblastically on a sterigma-like stalk. Stalks develop multilaterally and holoblastically from a conidiogenous cell, rarely proliferate sympodially, and measure 4–15 µm in length and 0.3–0.6 µm in width. Newly formed cells generally separate at the distal position of stalks. Occasionally chains and clusters of stalked conidia are formed. A sediment is formed. After one month at 17°C, a fragile pellicle and a sediment are present. There may be as many as 12 stalks on a cells.

Growth on YM agar: After one month at 17°C, the streak culture is grayish-orange, smooth, shining, soft to mucoid, and has an entire margin. Stalked conidia and ballistoconidia are formed.

Dalmau plate culture on corn meal agar: Mycelium and pseudomycelium are not formed.

Production of ballistoconidia: Ballistoconidia are produced on corn meal agar and YM agar. They are kidney-shaped or ellipsoidal, (1.3–5.3)×(2.1–9.2) µm, and formed on sterigma, which proliferate singly or in branches. The sterigmata occasionally become much longer than 30 µm (Fig. 400).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+/l	Ethanol	–
Sucrose	+	Glycerol	w/–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	w/–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–/l
Melezitose	+	Salicin	w/–
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	w
L-Arabinose	+	Citrate	w/–
D-Arabinose	+/l	Inositol	–/l
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	+	Gelatin liquefaction	w
D-Glucuronate	+	Acid production	–
D-Galacturonate	+	Thiamine-free	–
Starch formation	–	Growth at 33°C	–

Co-Q: 10 (Nakase et al. 1991b).
Mol% G+C: 49.5 (T_m); 47.7–48.4 (HPLC).
Xylose in cells: Present.

Origin of the strains studied: CBS 7552 (JCM 7824, IF0 10520), dead leaf of blady-grass (*Imperata cylindrica*), Nakase and Fungsin, Thailand; CBS 7553

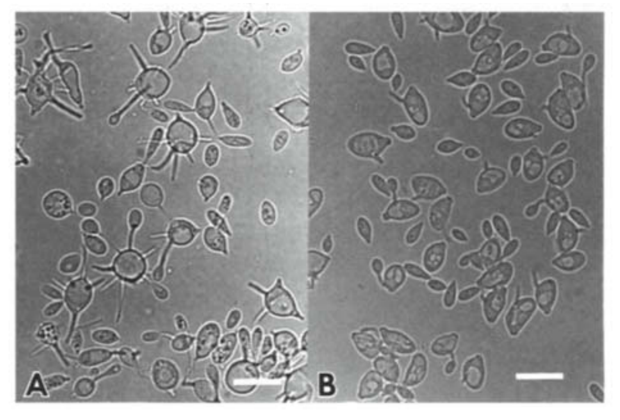


Fig. 400. *K. thailandica*, CBS 7552. (A) Vegetative reproduction by conidiation on stalks produced by parent cells after 3 days at 24°C on YM agar. (B) Ballistoconidia ejected from a colony on corn meal agar after 5 days at 22°C. Some ballistoconidia produce secondary ballistoconidia. Bars = 10 µm.

(JCM 7825, IF0 10521), dead leaf of rice plant (*Oryza sativa*), Nakase and Fungsin, Thailand.

Type strain: CBS 7552 (JCM 7824).

Comments on the genus

Nakase et al. (1991b) proposed the genus *Kockovaella* based on three strains isolated from leaves of plants in Thailand. This genus is characterized by the formation of three kinds of conidia: stalked conidia, ballistoconidia, and budding cells (blastoconidia); by the presence of xylose in the whole-cell hydrolyzate, and by ubiquinone Q-10. Partial sequences of 18S ribosomal RNA suggest a close phylogenetic relationship to *Fellomyces* species which produce neither ballistoconidia nor budding cells. The conidial characteristics of *Kockovaella* raise the question of the taxonomic significance of mode of conidiogenesis as a criterion for description of genera or higher taxa, and this issue was discussed by Nakase et al. (1991a).

99. *Kurtzmanomyces* Y. Yamada, M. Itoh, Kawasaki, Banno & Nakase

Y. Yamada and I. Banno

Diagnosis of the genus

Cells are globose, ovoidal to cylindrical. A single conidium is formed at the top of stalks and freed without forceful ejection at the distal end of the stalks. Occasionally stalks branch or elongate and additional conidia are formed. True mycelium is produced. Ballistoconidia are absent.

Glucose is not fermented. Nitrate is assimilated. D-Glucuronate and *myo*-inositol are not assimilated. Diazonium blue B color reaction is positive. Xylose is absent from the cells. Coenzyme Q-10 is present.

Type species

Kurtzmanomyces nectairei (Rodrigues de Miranda) Y. Yamada, M. Itoh, Kawasaki, Banno & Nakase

Species accepted

1. *Kurtzmanomyces nectairei* (Rodrigues de Miranda) Y. Yamada, M. Itoh, Kawasaki, Banno & Nakase (1988)
2. *Kurtzmanomyces tardus* Giménez-Jurado & van Uden (1990)

Key to species

1. a Trehalose assimilated .... *K. nectairei*: p. 780
   b Trehalose not assimilated .... *K. tardus*: p. 781

Systematic discussion of the species

99.1. *Kurtzmanomyces nectairei* (Rodrigues de Miranda) Y. Yamada, M. Itoh, Kawasaki, Banno & Nakase (1988a)

Synonyms:
Sterigmatomyces nectairei Rodrigues de Miranda (1975)
Fellomyces nectairei (Rodrigues de Miranda) Y. Yamada & Banno (1984a)

Growth in malt extract: After 3 days at 25°C, the cells are spheroidal to ovoidal, (2–7)×(4–8) µm, with 1–10 short stalks. Conidia develop laterally or terminally on stalks and branching of the stalks occurs (Fig. 401). Conidia separate at the distal end. After one month, stalks are branched and irregularly shaped (0.7–1)×(1–17) µm. A sediment is present.

Growth on malt extract agar: The cells are similar in size to those in malt extract; colonies are creamy, become salmon-colored by 5 days and orange-colored by one month. The texture is smooth, and the edges are entire.

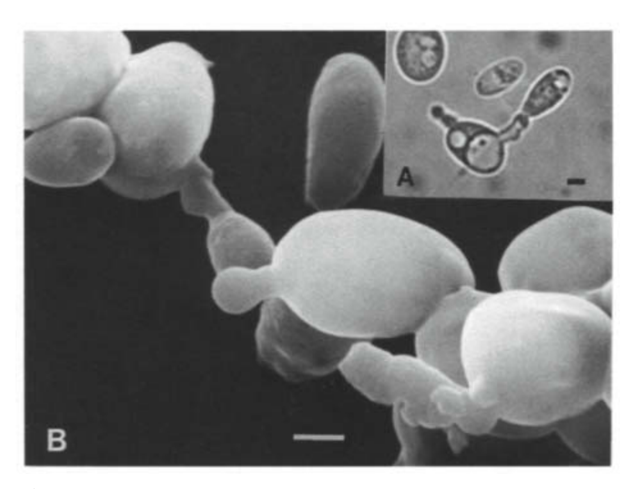


Fig. 401. *K. nectairei*, CBS 6405. After 72 hours at 25°C in malt extract. (A) Light microscopy. (B) Scanning electron microscopy. Bars = 1 µm.

Dalmau plate culture on corn meal agar: Within one month, short, sparse strands of true mycelium are present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	w
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	w
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	+	50% Glucose	–
Starch formation	–	0.01% Cycloheximide	–
Ethylamine	+	Growth at 30°C	–
Arbutin	–		

Co-Q: 10, CBS 6405 (Yamada et al. 1988a).
Mol% G+C: 52.5, CBS 6405 (BD: Kurtzman 1990a).
Xylose in cells: Absent.

Origin of the strain studied: CBS 6405 (IFO 10118), cheese, E.G. Dale, France.

Type strain: CBS 6405.

99.2. *Kurtzmanomyces tardus* Giménez-Jurado & van Uden (Giménez-Jurado et al. 1990)

The species description is from Giménez-Jurado et al. (1990).

Growth in glucose (2%)–yeast extract (0.5%)–peptone (1%) broth: After 3 days at 25°C, the cells are variable both in shape and size, spheroidal, ovoidal, ogival and obovate, measuring (3.0–5.0)×(4.0–8.0) μm. Reproduction occurs by the enteroblastic formation of 1–4 0.5–2.0 μm-long sterigmata per cell. Some of these sterigmata will eventually elongate to hypha-like outgrowths. Conidia separate distally from the parent cell. Mature conidia are ovate to clavate and may give rise to secondary cells. Quite often sterigmata branch to produce two distinct newly divided cells. Mycelial growth is produced sparingly in liquid medium, however, single cells with short and long hyphae are common. Growth is rather slow and after 1 week only a small sediment is formed.

Growth on glucose (2%)–yeast extract (0.5%)–peptone (1%) agar: After 1 week at 25°C, the streak culture is pale-orange to salmon-colored, smooth, soft, glossy, butyrous and the edges are entire. After 8 days or more, colonies are slightly raised, convex and sometimes have an elevated center. True mycelium develops abundantly without clamp connections.

Dalmau plate cultures on corn meal agar: Abundant formation of true mycelium consisting of slender, septate, branching hyphae without blastoconidia. Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Gelatin liquefaction	n
Saccharate	–	0.01% Cycloheximide	–
10% NaCl/5% glucose	–	Growth at 37°C	–

Co-Q: 10 (Giménez-Jurado et al. 1990).

Mol% G+C: 58.2, CBS 7421 (T_m: Giménez-Jurado et al. 1990).

Origin of the strain studied: The only known strain (CBS 7421, IGC 4529) was isolated from contaminated demineralized water in a laboratory, Oeiras, Portugal.

Type strain: CBS 7421.

Comments: This species grows slowly on common laboratory media. Giménez-Jurado et al. (1990) showed only 2.3% nDNA relatedness between the two species of *Kurtzmanomyces.*

Comments on the genus

Yamada and Banno (1984b) emended the genus *Sterigmatomyces* and described the new genus *Fellomyces.* They reserved *Sterigmatomyces* for anamorphic yeasts characterized by coenzyme Q-9 and by conidia borne on sterigmata which disjoint at septa formed in the mid-region of the sterigmata, and *Fellomyces* for anamorphic yeasts with coenzyme Q-10 and conidia which disjoint at distal septa in the sterigmata. Yamada et al. (1988b), based on the presence or absence of xylose in whole cell hydrolyzates, separated the newly created genus *Kurtzmanomyces* (xylose negative) with the single species *K. nectairei* from the genus *Fellomyces* (xylose positive). The validity of this generic rearrangement was confirmed by Yamada et al. (1989b) and Guého et al. (1990) based on rRNA sequence analyses. A second species of *Kurtzmanomyces* was described by Giménez-Jurado et al. (1990). Large subunit rRNA sequence analysis (J.W. Fell, personal communication) suggests the species to be congeneric. However, small subunit rRNA sequence analysis indicates that the two species are divergent (Yamada et al. 1991d).

100. *Malassezia* Baillon

D.G. Ahearn and R.B. Simmons

Diagnosis of the genus

Asexual reproduction is typically by monopolar (phialidic) enteroblastic budding on a broad base. The extruded bud is separated from the parent cell by a septum followed by fission. Elongate hyphal-like cells are rare in culture but true hyphae with clusters of blastospores may be commonly observed in skin scales from hosts (Fig. 402A). The formation and separation of the bud generally leaves a pronounced scar or collar from which later buds emerge (Fig. $402B_1$). Multipolar budding has been observed in one species (Fig. $402B_2$). The cell wall is multilamellar in nature, and the inner layer of the cell wall as observed with electron microscopy is corrugated with the grooves spiraling from the bud site (Fig. 402C). Chitin is concentrated in the portion of the cell wall forming the collar at the budding site.

Growth may require fatty acid (olive oil) supplementation. Growth occurs in enriched nutrient agars in the presence of 400 ppm cycloheximide. Sugars are not fermented. The urease and diazonium blue B reactions are positive. Coenzyme Q-9 is formed. Xylose is absent in whole-cell hydrolyzates.

Comments on physiological characterization of species of the genus: Because of their requirements for fatty acids (olive oil) and the fact that the fatty acid components of olive oil serve as sole sources of carbon for growth, standard sugar assimilation and fermentation tests are not applied.

Type species

Malassezia furfur (Robin) Baillon

Species accepted

1. *Malassezia furfur* (Robin) Baillon (1889)
2. *Malassezia pachydermatis* (Weidman) Dodge (1935)
3. *Malassezia sympodialis* Simmons & Guého (1990)

Key to species

1.	a	Lipophilic species, but show growth on enriched organic agars (e.g., Sabouraud's and glucose agars) without addition of oil	*M. pachydermatis*:	p. 782
	b	Obligatory lipophilic species; addition of long-chain fatty acids to culture media is required → 2		
2(1).	a	Cells occasionally show sympodial budding; insignificant growth with the addition of Tween 80 or oleic acid	*M. sympodialis*:	p. 783
	b	Cells budding exclusively percurrently; growth with the addition of Tween 80	*M. furfur*:	p. 782

Systematic discussion of the species

100.1. *Malassezia furfur* (Robin) Baillon (1889)

Synonyms:

Microsporon furfur Robin (1853)
Sporotrichum furfur (Robin) Saccardo (1886)
Pityrosporum furfur (Robin) Emmons, Binford & Utz (1970)
Saccharomyces ovalis Bizzozero (1884)
Malassezia ovalis (Bizzozero) Acton & Panja (1927)
Pityrosporum ovale (Bizzozero) Castellani & Chalmers (1913)
Saccharomyces sphaericus Bizzozero (1884) [non *Saccharomyces sphaericus* von Nägeli (1879)]
Pityrosporum malassezii Sabouraud (1904)
Cryptococcus malassezii (Sabouraud) Benedek (1930)
?*Pityrosporum orbiculare* Gordon (1951)

Growth on glucose–peptone–yeast extract agar with olive oil: After 3 days at 34°C, the cells are usually slightly ovoidal to ellipsoidal, (1.5–4.5)×(2.0–6.5) µm. Cells of some strains are mostly orbiculate and (2.5–4.5) µm in diameter; a few large cells (8–10 mm) may be observed (Figs. 402A, B_1, B_2, C). The streak culture is cream to yellowish, usually smooth, sometimes lightly wrinkled. The margin is typically entire, occasionally lobate. There is an essential requirement for olive oil or oleic acid for growth on malt or Sabouraud's agars. Some strains grow slowly with oleic acid. Optimum temperature for growth is near 34°C. Growth occurs at 37°C.

Mol% G+C: 66.0–66.7, four isolates (neotype CBS 1878, 66.7%) (T_m: Guého and Meyer 1989).

Origin of strains studied: Human skin scales (6); human blood (4); human ear (4); human lung tissue (1); human urine (1).

Type strain: CBS 7019.

100.2. *Malassezia pachydermatis* (Weidman) Dodge (1935)

Synonyms:

Pityrosporum pachydermatis Weidman (1925)
Pityrosporum canis Gustafson (1955)

Growth on glucose–peptone–yeast extract agar: After 3 days at 34°C, the cells are mostly ellipsoidal, (2.5–5.5)×(3.0–7.0) µm. The streak culture is cream to yellow, glistening to dull, smooth to rough, with an entire or lobate margin. Olive oil is not essential for growth but is

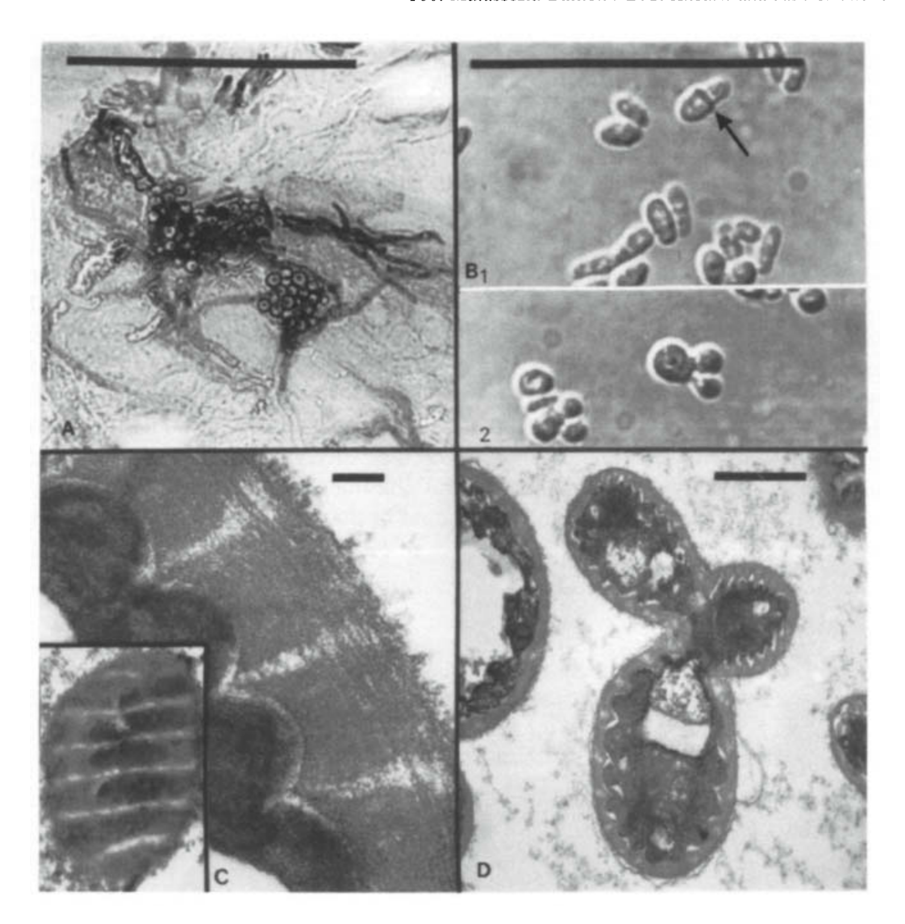


Fig. 402. (A) Clusters of budding yeast cells and hyphal elements of *M. furfur* in human skin. Bar = 100 μm. (B_1) Photomicrograph of *M. furfur* from glucose–peptone–yeast extract agar. Arrow indicates the collarette characteristic of the genus. (B_2) Photomicrograph of *M. sympodialis* showing sympodial budding. Bar = 50 μm. (C) TEM of *M. furfur* showing multilayered cell wall with corrugated inner surface. Bar = 0.1 μm. Inset: oblique section through cell showing spiral nature of the corrugations. (D) TEM of *M. sympodialis* showing characteristic cell wall and sympodial budding with continuity of cytoplasm between parent cell and buds. Bar = 1 μm.

stimulative for some strains. Glucose is assimilated slowly in yeast nitrogen base medium.

Mol% G+C: 55.1–56.1, five isolates; neotype (CBS 1879) 56.1 (T_m: Guého and Meyer 1989).

Origin of strains studied: Human sputum (1); human blood (5); human skin (7); human ear (1); human vagina (1); ear of dog (1).

Type strain: CBS 1879.

Comments: Some strains of this species do not grow, or only grow slowly, in yeast nitrogen base medium without the presence of olive oil. Slooff (1970c) reported that growth occurs with mannitol, glucitol, and occasionally with glycerol, lactic acid, and citric acid when tested with auxanogram plates. The species is most often associated with animals, particularly the ears of canines (Abou-Gabal et al. 1979, Guillot et al. 1995), but is also associated with humans (Simmons and Ahearn 1987).

100.3. *Malassezia sympodialis* Simmons & Guého (1990)

Growth on glucose–peptone–yeast extract agar with oil: After 3 days at 34°C, the cells are usually orbiculate and measure (2.5–7.5) × (2.0–8.0) μm. Asexual reproduction is mainly by monopolar budding, but sympodially budding cells may be observed (Fig. 402D). The streak culture is cream to yellowish, usually smooth and glistening. The margin is typically entire. Olive oil is essential for growth on enriched media, but no growth occurs when oleic acid is used as the fatty acid supplementation. Optimal growth occurs near 34°C. Growth occurs at 37°C.

Mol% G+C: 62.2±0.2, 13 isolates (Guillot and Guého 1995).

Origin of strains examined: Human skin (1); human ear (1).

Type strain: CBS 7222 is designated as the neotype. From human ear and tinea capitis.

Comments on the genus

The general characteristics of *M. sympodialis* are similar to those described for *Pityrosporum orbiculare* Gordon (1951). In the original description of *P. orbiculare,* Gordon (1951) noted that an essential requirement for fatty acids could not be satisfied by supplementation with oleic acid and that some cells reproduced by multipolar budding. Slooff (1970c) accepted the species in the genus noting its short viability in culture and a more stringent fatty acid requirement than observed for *P. ovale.* She examined only recently isolated material and indicated that multipolar budding did not occur, but that in some circumstances "budding or extrusion of a germ tube has been observed

from the free poles or from another locus on the cell wall." More recent reports of *P. orbiculare* appear to have relied only on globose cell shape for their identification of the species. Salkin and Gordon (1977) examined fresh isolates of *Pityrosporum* species with changing cell shape and fatty acid requirements and suggested, in agreement with others, that *P. ovale* and *P. orbiculare* were synonymous with *Malassezia furfur*. This synonymy was maintained by Yarrow and Ahearn (1984). The original type material and past isolates designated *P. orbiculare* (on the basis of inability to grow on oleic acid, as well as cell shape) are dead. Other isolates designated as *P. orbiculare* on the basis of globose cell shape have been found to represent *M. furfur* (Guého and Meyer 1989); therefore, *P. orbiculare* is continued as a synonym for *M. furfur*. The differences in mol% G+C between *M. furfur* (66%) and *M. sympodialis* (62%) and their low DNA relatedness clearly support their separate species status.

Polymerase chain reaction (PCR) fingerprinting studies have further confirmed the separation of *Malassezia* into the three described species accepted here (van Belkum et al. 1994). Guillot and Guého (1995) used ribosomal RNA sequence analysis and nuclear DNA comparisons to study diversity within the genus. The separate nature of *M. sympodialis* and *M. pachydermatis* was substantiated and phylogenetic groupings based on large subunit rRNA diversity indicated strains described as *M. furfur* to represent several species. More recently, Guého et al. (1996) described four new species of *Malassezia* on the basis of molecular divergence and have provided morphological and physiological comparisons that allow laboratory separation of the species using non-molecular criteria (Guillot et al. 1996).

101. *Moniliella* Stolk & Dakin

G.S. de Hoog and M.Th. Smith

Diagnosis of the genus

Colonies are smooth or velvety, grayish to olivaceous-black. Budding cells are ellipsoidal, and formed terminally on true hyphae which disarticulate at a later stage. Pseudomycelium and chlamydospores may be present. Cell walls are multilamellar. Septal dolipores are present.

Sugars are fermented. Nitrate is assimilated. Diazonium blue B reaction is positive. Urease is produced. Coenzyme Q-9 is produced. Xylose and fucose are absent from cell walls.

Type species

Moniliella acetoabutens Stolk & Dakin

Species accepted

1. *Moniliella acetoabutens* Stolk & Dakin (1966)
2. *Moniliella mellis* (Fabian & Quinet) V. Rao & de Hoog (1975)
3. *Moniliella pollinis* (Hennebert & Verachtert) de Hoog & Guého (1984)
4. *Moniliella suaveolens* (Lindner) von Arx (1972)

Key to species

See Table 83.

1. a Dark brown, thick-walled chlamydospores are present .... *M. acetoabutens*: p. 785
   b Chlamydospores are absent → 2
2(1). a Growth at 37°C .... *M. pollinis*: p. 787
   b Growth absent at 37°C → 3
3(2). a Sucrose assimilated .... *M. suaveolens*: p. 787
   b Sucrose not assimilated .... *M. mellis*: p. 786

Table 83
Key characters of species in the genus *Moniliella*

Species	Sucrose fermentation	Assimilation			Growth		Chlamydospores
		Galactose	Sucrose	Lactose	Vitamin-free medium	37°C	
Moniliella acetoabutens	+	–	+	+	+	+	+
M. mellis	–	+	–	–	–	–	–
M. pollinis	+	–	+	–	+	+	–
M. suaveolens	+	v	+	v	+	–	–

Systematic discussion of the species

101.1. *Moniliella acetoabutens* Stolk & Dakin (1966)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 15–19 mm in diameter, evenly felty, vaguely zonate, whitish, brownish-gray at the center, with dirty yellowish to blackish-brown reverse. Hyphae present, 3.5–4.5 μm wide, with intercalary, dark brown, thick-walled chlamydospores and with terminal chains of blastoconidia which finally disarticulate into rectangular arthroconidia (Fig. 403). Blastoconidia are (3.5–6.0)×(4.5–9.0) μm, subhyaline.

Fermentation:

Glucose	+	Maltose	+
Galactose	–	Lactose	–
Sucrose	+	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	n
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	+

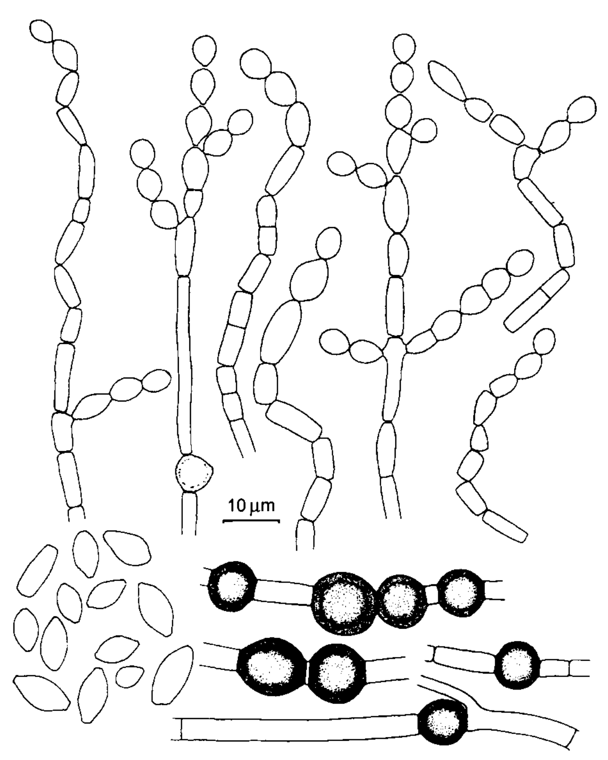


Fig. 403. *M acetoabutens*, CBS 169.66. Chains of blasto- and arthroconidia. Various media, 22°C, 10 days. CBS 594.68, liberated conidia. ChA, 22°C, 30 days. CBS 593.68, chlamydospores. ChA, 22°C, 30 days.

Additional assimilation tests and other growth characteristics:

Growth at 37°C +

Co-Q: 9, CBS 169.66.

Mol% G+C: 61.1–61.3, CBS 169.66, CBS 593.68 (T_m: de Hoog and Guého 1984).

Origin of the strains studied: CBS 169.66 (ATCC 18455), from sweet fruit sauce, Dakin, U.K.; from fruit sauce (1); from sweet pickle (1); from acetic acid containing product (2); from synthetic vinegar (1).

Type strain: CBS 169.66.

Comments: The species occurs nearly exclusively in substrates with low pH. It is easily distinguished from other *Moniliella* species by the consistent presence of intercalary chlamydospores in true hyphae, and hyaline or subhyaline conidia. In addition, *M. acetoabutens* does not ferment D-galactose but is able to grow at 37°C.

101.2. *Moniliella mellis* (Fabian & Quinet) V. Rao & de Hoog (1975)

Synonym:
Torula mellis Fabian & Quinet (1928)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 10 mm in diameter, cream-colored, gradually darkening to olivaceous-gray, dry, mat, finely velvety, cerebriform, and with a soft texture. Margins are sharp, slightly lobed. Odor is faintly fruity. Hyphae are 2.5–3.5 μm wide, subhyaline,

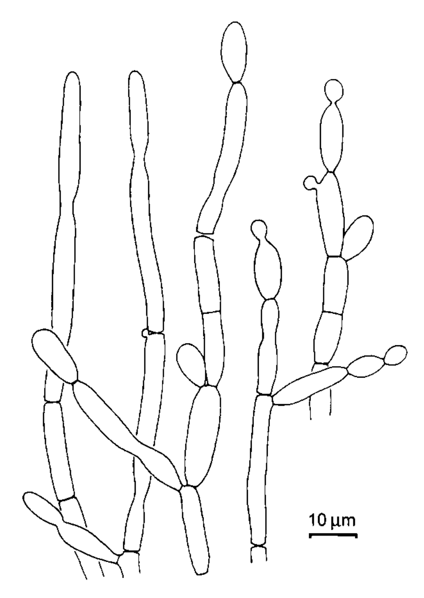


Fig. 404. *M. mellis*, CBS 350.33. Pseudomycelium with blasto- and arthroconidia. MEYA, 22°C, 5 days.

disarticulating into arthroconidia, and in a later stage locally forming short chains of blastoconidia. Conidia are subhyaline, ellipsoidal to cylindrical, variable in size, mostly about (4–6)×(10–25) μm (Fig. 404). Denticles are absent.

Fermentation:

Glucose	+	Maltose	+
Galactose	+	Lactose	–
Sucrose	–	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	–	Glycerol	n
Maltose	+	Erythritol	w
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	n
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	w
L-Arabinose	–	Citrate	w
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Urease	+	Growth at 37°C	–

Co-Q: 9, CBS 350.33.

Mol% G+C: 58.7, CBS 350.33 (T_m: de Hoog and Guého 1984).

Origin of the strain studied: CBS 350.33, from honey.

Type strain: CBS 350.33.

Comments: The species was preliminarily listed as a

synonym of *M. suaveolens* by de Hoog (1979a), which was confirmed by de Hoog and Guého (1984) on the basis of a similar mol% G+C of DNA. However, its source of isolation is quite peculiar. Additional differences are found in absence of utilization of sucrose (fermentative and assimilative) and absence of growth without vitamins. *Moniliella suaveolens* is a lipophilic species, whereas *M. mellis* is osmophilic. *Trichosporonoides nigrescens* Hocking & Pitt may be a synonym; see under the description of that species, p. 875.

101.3. *Moniliella pollinis* (Hennebert & Verachtert) de Hoog & Guého (1984)

Synonym:

Moniliella tomentosa (van Beyma) Stolk & Dakin var. *pollinis* Hennebert & Verachtert (Dooms et al. 1971)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 3 mm in diameter, dry, grayish-white, and farinose with strong transverse fissures. The margin is sharp and lobed. Odor is insignificant. Hyphae are about 3 µm wide, hyaline or subhyaline, and disarticulate into arthroconidia; hyphae terminally change into pseudomycelium and produce apical chains of blastoconidia. Conidia are hyaline or subhyaline, ellipsoidal, (3.5–5.0)×(9–15) µm, continuous or with a median septum; short-cylindrical denticles often remain on supporting cells (Fig. 405).

Fermentation:

Glucose	+	Maltose	+
Galactose	w	Lactose	–
Sucrose	+	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	n
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	n
Soluble starch	v	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	n
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

Urease	+	Growth at 37°C	+

Co-Q: 9, CBS 461.67.

Mol% G+C: 50.4, CBS 461.67 (T_m: de Hoog and Guého 1984).

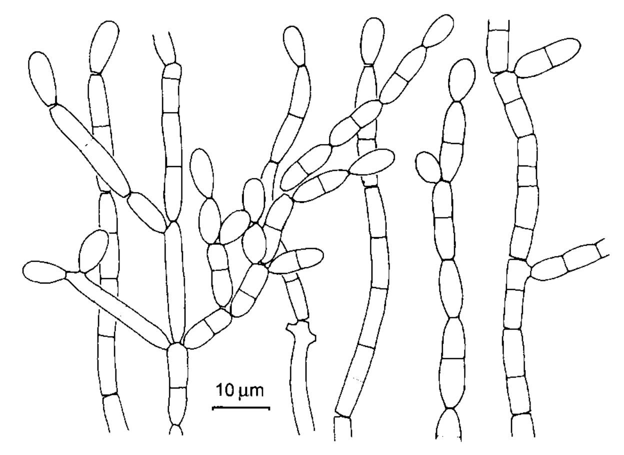


Fig. 405. *M. pollinis*, CBS 461.67. Pseudomycelium with blasto- and arthroconidia. PCA, 22°C, 10 days.

Origin of the strain studied: CBS 461.67 (MUCL 11525), from pollen in honey comb, Hajny, U.S.A.

Type strain: CBS 461.67.

Comments: *M. pollinis* was recognized as a separate species by de Hoog and Guého (1984) on the basis of a mol% G+C of DNA which was considerably lower than that of *M. suaveolens*. Presence of growth at 37°C is usable as a key feature. It was the only strain isolated from fresh pollen. The type strain is now degenerative in that the arthroconidial form of propagation is prevalent.

101.4. *Moniliella suaveolens* (Lindner ex Lindner) von Arx (1972)

Synonyms:

Sachsia suaveolens Lindner (1895)
Oospora suaveolens (Lindner) Saccardo & D. Saccardo (1906)
Candida suaveolens (Lindner) Langeron & Guerra (1938)
Geotrichum suaveolens (Lindner) Ciferri (Diddens & Lodder 1942)
Cladosporium suaveolens (Lindner) Delitsch (1943)
Sachsiella suaveolens (Lindner) Ciferri (1955a)
Monilia nigra Burri & Staub (1909)
Torula nigra (Burri & Staub) Saccardo & Trotter (1911)
Moniliella suaveolens (Lindner) von Arx var. *nigra* (Burri & Staub) de Hoog (1979a)
Torula dematia Berkhout (1923)
Monilia cerebriforme van Beyma (1933)
Monilia macrospora van Beyma (1933)
Monilia microspora van Beyma (1933)
Monilia tomentosa van Beyma (1933)
Moniliella tomentosa (van Beyma) Stolk & Dakin (1966)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 4–12 mm in diameter, quite variable, grayish-white, olivaceous-green to olive-black with a light marginal zone and velvety or cerebriform, with butyrous texture. The margin is mostly sharp and lobed. Odor is faintly vinaceous or cheese-like. Hyphae are 1.5–3.5 µm wide, hyaline to pale olivaceous and they disarticulate into arthroconidia; hyphae terminally change into pseudomycelium and produce apical chains of blastoconidia. Conidia are subhyaline to olivaceous-brown, ellipsoidal, (3–6)×(6–19) µm, and finally thick-walled; short-cylindrical denticles often remain on supporting cells (Fig. 406).

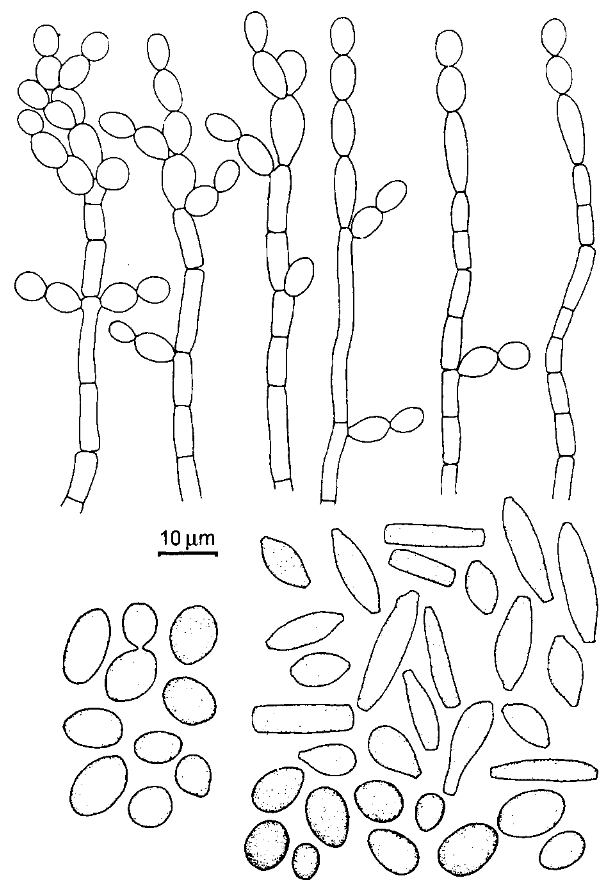


Fig. 406. *M. suaveolens*. Various strains on PCA, 22°C. Branched chains of blasto- and arthroconidia, 5 days; liberated conidia in various stages of pigmentation, 1 month.

Fermentation:

Glucose	+	Maltose	+
Galactose	v	Lactose	v
Sucrose	+	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	v
Trehalose	–	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	v	α-Methyl-D-glucoside	–
Melezitose	v	Salicin	v
Inulin	–	D-Gluconate	n
Soluble starch	v	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

Urease	+	Growth at 37°C	–

Co-Q: 9, CBS 126.42.

Mol% G+C: 58.1–61.7, 5 strains (T_m: de Hoog and Guého 1984).

Origin of the strains studied: CBS 126.42, from mash syringe, Lindner, Germany; CBS 220.32, type strain of *Monilia cerebriforme,* from tobacco; CBS 221.32, type strain of *Monilia macrospora,* from tobacco; CBS 223.32, type strain of *Monilia microspora,* from tobacco; CBS 224.32, type strain of *Monilia tomentosa,* from tobacco; from milk (1); from margarine and butter (19); from tobacco (1); from cheese (4); from spoiled muffin (1); from human sputum (1).

Type strain: CBS 126.42.

Comments: The species is extremely variable in its morphology (de Hoog 1979a) as well as in its physiology (Martínez et al. 1979). Key features are the olivaceous pigments in nearly all cultures, the relatively large conidia often borne on protruding scars, fermentation of D-glucose, sucrose, maltose and mostly D-galactose, absence of growth with L-sorbose, trehalose and inositol and at 37°C. In addition, the fruity fragrance is remarkable.

Colonies may become hyphal due to long-term maintenance on artificial media. Such cultures mostly show more expanding growth, are velvety rather than butyrous and show less pigmentation. For practical reasons, de Hoog (1979a) maintained a separate variety for such variants, which, however, is taxonomically insignificant. The few strains from pollen and honey, listed by de Hoog (1979a) under *M. suaveolens,* are treated here as separate taxa.

Nearly all strains were isolated from fatty substrates such as cheese, butter, margarine, and bakery products; some came from tobacco. Isolates sometimes proved to be resistant to food preservatives, such as sorbinic acid. Two reports have been published of strains that were pathogenic in mammals. The human case (Kocková-Kratochvílová et al. 1987) concerned a mixed superficial infection. McKenzie et al. (1984) reported two cases from cats, but the described strains are atypical for *M. suaveolens*. It may be supposed that the low maximum growth temperature of *M. suaveolens* prevents serious infections.

The species is of basidiomycetous affinity, as demonstrated by lamellar cell walls and dolipore septa (Martínez 1979). Teleomorphs are unknown. Large numbers of mating experiments were unsuccessful (de Hoog, unpublished). On acidic media, some strains show very large, erect, clavate hyphae, terminally provided with variously shaped conidium-like inflations. The karyology of these structures has not yet been determined.

102. *Phaffia* M.W. Miller, Yoneyama & Soneda

M.W. Miller and H.J. Phaff

Diagnosis of the genus

Vegetative cells reproduce by enteroblastic budding, are ellipsoidal and occur singly, in pairs, and occasionally in short chains. Carotenoid pigments are synthesized (mainly astaxanthin and a minor proportion of β-carotene) which give cells *en masse* a red to salmon-red color. True mycelium is absent, but a rudimentary pseudomycelium may be present. Chlamydospores are formed. A pellicle is formed on liquid media.

Fermentative ability is present. Starchlike compounds are formed (synthesis is pH independent). Inositol is not assimilated as sole carbon source. Nitrate is not assimilated. Coenzyme Q-10 is present. Diazonium blue B and urease reactions are positive. Xylose is present in whole-cell hydrolyzates.

Type species

Phaffia rhodozyma M.W. Miller, Yoneyama & Soneda

Species accepted

1. *Phaffia rhodozyma* M.W. Miller, Yoneyama & Soneda (1976)

Systematic discussion of the species

The description of *Phaffia rhodozyma* is given under its teleomorph *Xanthophyllomyces dendrorhous*: p. 718.

Comments on the genus

Phaffia rhodozyma was tentatively described under the name *Rhodozyma montanae* by Phaff et al. (1972). This binomial is a *nomen nudum,* because a Latin description was not provided. Miller et al. (1976b) renamed the yeast *Phaffia rhodozyma,* provided a Latin description and gave a more detailed listing of its physiological and morphological properties. They also compared it with *Rhodomyces dendrorhous,* a yeast superficially described by Ludwig (1891, 1896). Apparently, Ludwig never isolated this organism in pure culture, and as a consequence, it is not possible to determine whether *P. rhodozyma* and *R. dendrorhous* represent the same species. Because the type strain of *P. rhodozyma* is conspecific with *Xanthophyllomyces dendrorhous* (Golubev 1995), *P. rhodozyma* is regarded as the anamorph of *X. dendrorhous.*

Phaffia appears to have a restricted geographic distribution. Nine strains were isolated in mountainous locations in a wide area of Japanese islands and a single strain came from Alaska, U.S.A., all from exudates of broad-leafed trees (Miller et al. 1976b). Golubev et al. (1977a) isolated 67 additional strains in Russia from birch fluxes and bark during a seven year survey. One additional strain was obtained from Turpeinen, who isolated it from birch sap in Finland. Although numerous surveys have been published on tree associated yeasts in many parts of the world (reviewed by Phaff and Starmer 1987) no other isolations of *Phaffia rhodozyma* have been reported.

Because of its high astaxanthin content, *Phaffia rhodozyma* has been used as a dietary pigment source to impart the desirable orange-red color to the normally white flesh of pen-reared salmonids and crustaceans (Johnson et al. 1977). Because the pigment of untreated cells is only poorly absorbed by fish, an enzymatic procedure for improving the extractability of astaxanthin was developed by Johnson et al. (1979). To increase astaxanthin yields, An et al. (1989) were able, by mutagenesis, to derive highly pigmented mutant strains of *Phaffia rhodozyma* with greatly increased astaxanthin content.

103. *Pseudozyma* Bandoni emend. Boekhout and a comparison with the yeast state of *Ustilago maydis* (De Candolle) Corda

T. Boekhout and J.W. Fell

Diagnosis of the genus

Yeast cells are variable in shape, ovoidal, ellipsoidal or cylindrical; hyphae occur, usually with retraction septa and with the cytoplasm in cells separated by lysed cells; fusiform blastoconidia occur on sterigma-like denticles and may form an aerial mycelium made up of branched, acropetal chains of conidia; chlamydospores may be present, but ballistoconidia are absent. Colonies are whitish, pinkish, orange, or brownish-yellow, usually dimorphic with the central part yeastlike, and the margin fringed with hyphae. A thin aerial mycelium is usually present, which toward the margin may become zonate. Clamp connections are absent.

Sugars are not fermented. Diazonium blue B and urease reactions are positive. *myo*-Inositol and D-glucuronate are assimilated, and extracellular starch-like compounds are not produced. Xylose is absent from whole-cell hydrolyzates. The major ubiquinone is Q-10.

Species accepted

1. *Pseudozyma antarctica* (S. Goto, Sugiyama & Iizuka) Boekhout (1995)
2. *Pseudozyma aphidis* (Henninger & Windisch) Boekhout (1995)
3. *Pseudozyma flocculosa* (Traquair, Shaw & Jarvis) Boekhout & Traquair (1995)
4. *Pseudozyma fusiformata* (Buhagiar) Boekhout (1995)
5. *Pseudozyma prolifica* Bandoni (1985)
6. *Pseudozyma rugulosa* (Traquair, Shaw & Jarvis) Boekhout & Traquair (1995)
7. *Pseudozyma tsukubaënsis* (Onishi) Boekhout (1995)
8. *Ustilago maydis* (De Candolle) Corda (1842)

Key to species

See Table 84.

1. a Galactose assimilated → 2
   b Galactose not assimilated . . . . . . *P. fusiformata*: p. 793; *Ustilago maydis*: p. 796

2(1). a Lactose assimilated → 3
   b Lactose not assimilated → 6

3(2). a Melebiose assimilated . . . . . . *P. aphidis*: p. 792
   b Melebiose not assimilated → 4

4(3). a Ribitol assimilated → 5
   b Ribitol not assimilated . . . . . . *P. tsukubaënsis*: p. 795

5(4). a Salicin assimilated . . . . . . *P. antarctica*: p. 791
   b Salicin not assimilated . . . . . . *P. prolifica*: p. 794

6(2). a L-Rhamnose assimilated . . . . . . *P. rugulosa*: p. 794
   b L-Rhamnose not assimilated . . . . . . *P. flocculosa*: p. 792

Table 84
Key characters of species in the genus *Pseudozyma*

Species	Assimilation				
	Galactose	Lactose	Melibiose	L-Rhamnose	Salicin
Pseudozyma antarctica	+	+	–	v	+
P. aphidis	+	+	+	+	+
P. flocculosa	+	–	+	–	+
P. fusiformata	–	–	v	–	v
P. prolifica	+	+	–	+	–
P. rugulosa	+	–	+	+	+
P. tsukubaënsis	+	+	–	–	–
Ustilago maydis	–	+	–	+	+

Systematic discussion of the species

103.1. *Pseudozyma antarctica* (S. Goto, Sugiyama & Iizuka) Boekhout (1995)

Synonyms:

Sporobolomyces antarcticus S. Goto, Sugiyama & Iizuka (1969)
Candida antarctica nom. nud., as *C. antarctica* (S. Goto, Sugiyama & Iizuka) Kurtzman, Smiley, Johnson & Hoffman (Meyer et al. 1984)
Vanrija antarctica (S. Goto, Sugiyama & Iizuki) R.T. Moore (1987a)
Trichosporon oryzae Ito, Iizuka & Sato (1974)

Growth on 5% malt extract agar: After 5 days at 17°C, cells are cylindrical to fusiform, variable in size, (5–35)×(1.5–3.0) μm, with 1–3 oil droplets, and single. Conidiogenesis is polar on short denticles and with sympodial proliferation (Fig. 407). Hyphae are abundant with the cytoplasm retracted in cells, (25–60)×(1.5–2.2) μm, and with sterigmata on which fusiform blastoconidia are formed. Colonies are dimorphic, with the center flat to somewhat raised, glabrous to velvety hirsute, smooth to somewhat irregularly furrowed, dull, pale creamish-white to yellowish-brown. Growth near the margin is zonate, somewhat hairy to velutinous, and with the margin fringed.

Growth on the surface of assimilation media (glucose): A ring, floating flocks and a sediment are formed.

Dalmau plate culture on morphology agar: After 5 days at 17°C, extensive hyphae may be formed with the cytoplasm retracted. Yeastlike cells are ellipsoidal, (4.0–10.0)×(2.0–4.0) μm, with polar conidiogenesis and sympodial proliferation. Aerobic growth is whitish to pinkish-cream, dull, with the surface smooth, somewhat pustulate to reticulate, and covered with hyphal fascicles or tiny hairs. The aerial mycelium is made up of ramifying acropetal chains of fusiform blastoconidia being formed on sterigmata.

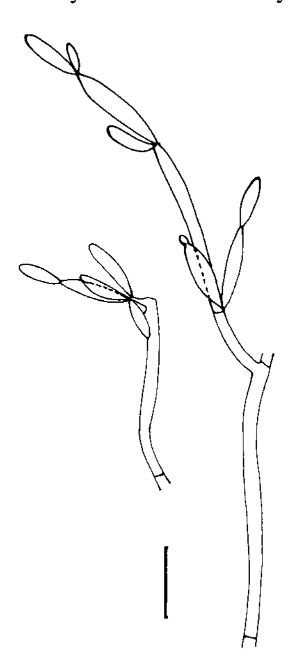


Fig. 407. *P. antarctica*, CBS 214.83. Hyphae and chains of blastoconidia. Yeast morpholgy agar, 5 days, 25°C. Bar = 10 μm.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	v	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
Saccharate	–	Nitrite	+
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose	–	Growth at 37°C	v
Starch formation	–		

Co-Q: 10 (Boekhout et al. 1992b).

Mol% G+C: 61.2–61.6 (BD: Kurtzman 1990a).

Cell wall hydrolyzates: Glucose, mannose low, galactose and glucosamine (Weijman 1979b, Weijman and Rodrigues de Miranda 1988, Boekhout et al. 1992b).

Origin of the strains studied: CBS 214.83, type strain of *Sporobolomyces antarcticus,* from lake sediment, J. Sugiyama, Lake Vanda, Victoria Land, Antarctica; CBS 516.83, type strain of *Trichosporon oryzae,* from unpolished new crop rice, H. Ito, Japan.

Type strain: CBS 214.83.

Comments: The species *Sporobolomyces antarcticus, Trichosporon oryzae* and *Sterigmatomyces aphidis* have been considered conspecific because of high DNA–DNA binding values (Kurtzman 1990a). For the first two species this is also supported by nucleotide sequence analysis, as their partial 26S ribosomal DNA sequences were found to be identical (Boekhout et al. 1995a). However, the partial 26S ribosomal DNA sequence of *Sterigmatomyces aphidis* was found to be slightly different, and therefore this species is maintained as separate in this treatment. The basidiomycetous nature of these fungi is supported by positive diazonium blue B and urease reactions. Phylogenetic affinity with the Ustilaginales is supported by morphology (Boekhout 1987), the presence of a narrow pore-like structure between the conidia (Boekhout, unpublished data) and partial 26S rDNA nucleotides (Boekhout et al. 1995a). Arthroconidia as they occur in *Trichosporon* are not formed. It seems likely that hyphal cells in which the cytoplasm retracts may have

been interpreted as arthoconidia (see Ito et al. 1974). *Trichosporon oryzae* was reported to be highly resistant to gamma radiation (Ito et al. 1974). The suggested synonymy of *Trichosporon oryzae* with *Candida edax* (Jong and King 1977b) is not accepted, as this latter species is the anamorph of *Stephanoascus smithiae* (Ascomycetes), p. 402 (Giménez-Jurado et al. 1994).

103.2. *Pseudozyma aphidis* (Henninger & Windisch) Boekhout (1995)

Synonym:

Sterigmatomyces aphidis Henninger & Windisch (1975b)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are fusiform, variable in size, (5.0–33.0)×(1.8–3.0) µm, and contain many oil droplets. Budding is polar on short denticles. Hyphae are present with the cytoplasm retracted in the cells, (30–50)×(2.0–3.0) µm, and with sterigmata on which cylindrical to fusiform blastoconidia, (20–36)×(2.0–2.7) µm, are formed. Colonies are dimorphic, flat, velvety-velutinous, dull, whitish to pale yellowish-cream, zonate towards the margin, and with the margin fringed.

Growth on assimilation media (glucose): A ring, floating flocks, a film and a sediment are present.

Dalmau plate culture on morphology agar: After 5 days at 17°C, hyphae are abundant. Aerobic growth is whitish, dull, velvety-velutinous, flat, with the center somewhat pustulate, and the margin fringed. The aerial mycelium consists of ramifying and acropetal chains of fusiform blastoconidia.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	–
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	+
Cellobiose	s	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	s
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
Saccharate	+	Urease	+
D-Glucuronate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 37°C	+

Co-Q: 10.

Mol% G+C: 61.5 (BD: Kurtzman 1990a).

Cell wall hydrolyzates: Not determined.

Origin of the strain studied: CBS 517.83, type strain of *Sterigmatomyces aphidis,* from secretion of Aphididae on leaves of *Solanum pseudocapsicum,* W. Henninger and S. Windisch, Germany.

Type strain: CBS 517.83.

Comments: *Pseudozyma aphidis* was suggested to be conspecific with *Pseudozyma antarcticus* and *Trichosporon oryzae* (Kurtzman 1990a). However, based on the 64–68% DNA–DNA similarity between *P. aphidis* and the remaining two species (Kurtzman 1990a), partial 26S ribosomal DNA sequences, and the presence of physiological differences, we regard *P. aphidis* as a separate species. The suggestion that this species belongs to the Ustilaginales (W. Henninger, unpublished data; Boekhout 1987) was confirmed by partial 26S rDNA sequences (Boekhout 1994, Boekhout et al. 1995a). The assimilation of methanol, ethanol, and galactitol, and growth in vitamin-free medium (Henninger and Windisch 1975b) were not confirmed by the present study.

103.3. *Pseudozyma flocculosa* (Traquair, Shaw & Jarvis) Boekhout & Traquair (Boekhout 1995)

Synonyms:

Stephanoascus flocculosus Traquair, Shaw & Jarvis (1988b)

Sporothrix flocculosa Traquair, Shaw & Jarvis (1988b)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are fusiform, variable in size and with many oil droplets. Two size classes appear apparent, viz. long elements, measuring (13–35)×(2.0–3.0) µm and shorter elements of (3.0–7.0)×(1.5–3.0) µm. Conidiogenesis is polar, on short denticles and with sympodial proliferation. Hyphae, which measure (25–37)×(1.8–2.2) µm, are characterized by retracted cytoplasm. The hyphae have sterigmata on which fusiform blastoconidia are formed. Colonies are flat with the center somewhat warty, dull, whitish, and finely pruinose, but toward the margin they are somewhat zonate, finely whitish pruinose and with the margin fringed.

Growth on the surface of assimilation media: Floating flocks, a thick film and a sediment are formed.

Dalmau plate culture on morphology agar: After 5 days at 17°C, the cells are mostly lysed. Aerobic growth is whitish, dull and flat with the center warty to somewhat pustulate and covered with a thin aerial mycelium. Hyphae have the cytoplasm retracted in the cells and measure (20–65)×(1.5–2.0) µm. The hyphae are characterized by sterigmata on which ramifying acropetal chains of fusiform conidia occur, usually made up of two rows of long cylindrical to fusiform cells, (20–40)×(2.0–2.5) µm, and with a terminal whorl of shorter fusiform blastoconidia, (3.0–9.0)×(2.0–3.0) µm.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	–
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	+
Cellobiose	s	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	s	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	s	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	s	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
Saccharate	–	Nitrite	+
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose	–	Growth at 37°C	–
Starch formation	–		

Co-Q: Not determined.
Mol% G+C: Not determined.
Cell wall hydrolyzates: Not determined.

Origin of the strain studied: CBS 167.88, leaf of clover (*Trifolium pratense*) infected with mildew (*Erisyphe polygoni*), J.A. Traquair, Ontario, Canada.

Type strain: CBS 167.88.

Comments: *Stephanoascus flocculosus* was described as a homothallic ascomycete forming thick, persistent asci with 2–4 galeate ascospores (Traquair et al. 1988b). However, this observation could not be confirmed by the present authors. The ascus-like structures illustrated by Traquair et al. (1988b) are probably chlamydospores. At present, there is substantial evidence that this fungus represents a heterobasidiomycete. The morphological appearance of the colonies, and the presence of branched chains of ramoconidia (Traquair et al. 1988b) is similar to those occurring in anamorphs of Ustilaginales (Boekhout 1987). The basidiomycetous nature of this fungus is supported by positive diazonium blue B and urease reactions, as well as partial nucleotide sequences of the 26S ribosomal DNA (Boekhout 1994, Boekhout et al. 1995a, Kurtzman and Robnett 1995). Molecular data support a close phylogenetic affiliation with the Ustilaginales, viz. *Ustilago maydis* (Boekhout et al. 1995a). Contrary to the data of Traquair et al. (1988b), we did not observe assimilation of lactose and ethanol. The species has potential to antagonize cucumber powdery mildew (*Sphaerotheca fuliginea*) (Jarvis et al. 1989).

103.4. *Pseudozyma fusiformata* (Buhagiar) Boekhout (1995)

Synonym:
Candida fusiformata Buhagiar (1979)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are cylindrical, ellipsoidal to fusiform, (4.5–8.5)×(1.0–4.0) μm, single, and usually contain 1–2 oil droplets. Conidiogenesis is polar and either sessile or on a short denticle. Short hyphae are present. Colonies are butyrous, smooth to wrinkled, glabrous, shiny, whitish, cream, light salmon to vinaceous-buff, and with the margin entire.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 5 days at 17°C, short hyphal cells may occur. Hyphae usually are for the greater part lysed and have retraction septa. Aerobic growth is pinkish-cream to salmon, shiny, weakly venose or reticulate, and with the margin entire, straight or lobate. A thin aerial mycelium occurs, made up of acropetal chains of fusiform blastoconidia.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	s
Cellobiose	s	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	s
D-Arabinose	+	Inositol	+
D-Ribose	s	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	v	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
Saccharate	–	Nitrite	+
D-Glucuronate	+	Growth at 25°C	+
50% (w/w) Glucose	–	Growth at 30°C	s
Starch formation	–	Growth at 37°C	–

Co-Q: Not determined.
Mol% G+C: 58.1 (T_m: Buhagiar 1979).
Cell wall hydrolyzates: Mannose low (Weijman et al. 1988).

Origin of the strains studied: CBS 6232, leaves of barley, H. Fokkema, Netherlands; CBS 6951, cauliflower (*Brassica oleacea* var. *botrytis*), R.W.M. Buhagiar, Great Britain.

Type strain: CBS 6951.

Comments: The positive urease and diazonium blue B reactions clearly indicate the basidiomycetous nature of

this species, as has been suggested by Weijman et al. (1988). This fungus does not fit the current definitions of the anamorphic genera *Cryptococcus* and *Rhodotorula* because of its inability to synthesize extracellular starch-like compounds and its ability to assimilate *myo*-inositol. Partial 26S ribosomal DNA sequences strongly suggest that this species is an anamorph of the Ustilaginales (Boekhout et al. 1995a), which is supported by morphology, viz. the presence of an aerial mycelium made up of branched, acropetal chains of fusiform blastoconidia.

103.5. *Pseudozyma prolifica* Bandoni (1985)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are fusiform, variable in size, (7–50)×(1.5–2.5) μm, and contain many oil droplets. Conidiogenesis is polar, and on short denticles. Hyphae occur, with the cytoplasm retracted in cells, and measure (17–30)×(2.0–3.5) μm (ca. 1.0 μm wide hyphae occur as well). Sterigmata occur on which fusiform blastoconidia are formed. Colonies are dimorphic with the center somewhat raised and warty, dull, whitish to cream, and covered with fine hairs. Near the margin, growth is flat, finely hirsute, and with the margin fringed.

Growth on the surface of assimilation media (glucose): Floating flocks and a sediment are formed.

Dalmau plate culture on morphology agar: After 5 days at 17°C, hyphae are abundant, have the cytoplasm retracted in cells, and measure (24–50)×(2.0–3.0) μm. The hyphae have retraction septa and sterigmata on which fusiform blastoconidia, (12–37)×(2.0–3.0) μm in size, are formed. Aerobic growth is whitish, velvety-velutinous, flat to somewhat pustulate, and with the margin fringed.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	s
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
Saccharate	–	Nitrite	+
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose	–	Growth at 37°C	–
Starch formation	–		

Co-Q: Not determined.
Mol% G+C: Not determined.
Cell wall hydrolyzates: Not determined.

Origin of the strain studied: CBS 319.87, litter of *Scirpus microcarpus,* growing in a seepage area on a steep hill side, Vancouver, R.J. Bandoni, B.C., Canada.

Type strain: CBS 319.87.

Comments: *Pseudozyma prolifica* has been described as a pleomorphic hyphomycete forming scolecoconidia, stauroconidia, blastoconidia, arthroconidia and chlamydospores (Bandoni 1985). This author noted similarities of the conidia to those of species of aquatic hyphomycetes, including species of *Articulospora, Varicosporium, Dendrospora* and *Tricladium,* but did not suggest a relationship for the taxon. However, the culture CBS 319.87 of *Pseudozyma prolifica,* deposited in the CBS collection, morphologically strongly resembles anamorphs of Ustilaginales. The basidiomycetous nature of *P. prolifica* is supported by positive diazonium blue B and urease reactions, and partial 26S ribosomal DNA nucleotide sequences (Boekhout et al. 1995a).

103.6. *Pseudozyma rugulosa* (Traquair, Shaw & Jarvis) Boekhout & Traquair (Boekhout 1995)

Synonyms:
Stephanoascus rugulosus Traquair, Shaw & Jarvis (1988b)
Sporothrix rugulosa Traquair, Shaw & Jarvis (1988b)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ellipsoidal to fusiform, (8.0–20)×(2.0–2.5) μm, and with many oil droplets. Budding is polar on short denticles. Hyphae occur with the cytoplasm retracted in the cells, (25–42)×(1.8–2.5) μm, and have sterigmata on which ramifying chains of fusiform blastoconidia are formed.

Growth on the surface of assimilation media: A ring, floating flocks and a sediment are present.

Dalmau plate culture on morphology agar: After 5 days at 17°C, the cells grow poorly. Aerobic growth is pale yellowish-brown, dull, flat, venose to somewhat pustulate, covered with fine hairs, and with the margin entire to somewhat fringed. The aerial mycelium consists of ramifying acropetal chains of fusiform blastoconidia of variable shape, (5.0–20.0)×(1.8–3.0) μm,

gradually becoming smaller towards the end of the chain.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	w
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	s
Cellobiose	s	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	s	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	s
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	w

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
Saccharate	+	Urease	+
D-Glucuronate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 37°C	+

Co-Q: Not determined.
Mol% G+C: Not determined.
Cell wall hydrolyzates: Not determined.

Origin of the strain studied: CBS 707.88, leaf of maize (*Zea mays*) infected with molds, J.A. Traquair, Ontario, Canada.

Type strain: CBS 170.88.

Comments: *Stephanoascus rugulosus* was described as a homothallic ascomycete, forming globose asci with 2–4 galeate ascospores (Traquair et al. 1988b). However, the ascomycetous nature of this fungus was not confirmed by the present authors. On the contrary, there is substantial evidence that this fungus belongs to the Ustilaginales (Heterobasidiomycetes). This is supported by positive diazonium blue B and urease reactions, partial nucleotide sequences of the 26S ribosomal DNA (Boekhout 1994, Boekhout et al. 1995a, Kurtzman and Robnett 1995) and the mode of conidiogenesis, viz. the formation of acropetally and ramifying chains of fusiform blastoconidia (see Traquair et al. 1988b, Boekhout 1987). In our opinion the ascus-like structures, as depicted by Traquair et al. (1988b), were chlamydospores with oil droplets. The reported assimilation of lactose (Traquair et al. 1988b) could not be confirmed. *Pseudozyma rugulosus* has an antagonistic capacity against cucumber powdery mildew (Jarvis et al. 1989).

103.7. *Pseudozyma tsukubaënsis* (Onishi) Boekhout (1995)

Synonyms:
Candida tsukubaënsis Onishi (1972)
Vanrija tsukubaënsis (Onishi) R.T. Moore (1987a)
Cryptococcus tsukubaënsis (Onishi) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are ellipsoidal, (5.0–15)×(2.8–4.2) μm, with 2–4 oil droplets, and single. Budding is polar and either sessile or on short denticles. Hyphae occur, with the cytoplasm retracted in cells and measure (10–80)×(1.0–3.0) μm. Sterigmata occur on which cylindrical to fusiform blastoconidia are formed. Colonies are dimorphic, flat, with the central part yeastlike, glabrous, smooth to somewhat warty, and the margin flat and covered with acute hyphal fascicles, and toward the margin, growth becomes hirsute, dull, pale yellowish-brown, and with the margin fringed.

Growth on the surface of assimilation media (glucose): A ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 5 days at 17°C, hyphae are formed, which are lysed for the most part. Aerobic growth is grayish-cream, dull, hirsute and with the margin fringed. Hyphae occur with the cytoplasm retracted in cells and with sterigma near the septa. Allantoidal blastoconidia, (12–23)×(2.5–3.5) μm, occur on sterigmata, short denticles or are sessile on the hyphae. A thin aerial mycelium is made up of ramifying, acropetal chains of fusiform blastoconidia.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	s	Inositol	+
D-Ribose	s	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	s	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
Saccharate	–	Nitrite	+
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose	–	Growth at 37°C	–
Starch formation	–		

Co-Q: Not determined.
Mol% G+C: 53.3 (T_m: Barnett et al. 1990).
Cell wall hydrolyzates: Mannose, galactose, glucose, mannitol, inositol and probably arabinitol (H. Roeijmans and T. Boekhout, unpublished results).

Origin of the strain studied: CBS 6389, flower, H. Onishi, Japan.
Type strain: CBS 6389.

Comments: The positive diazonium blue B and urease tests clearly indicate the basidiomycetous nature of this species. The ability to assimilate *myo*-inositol and the inability to synthesize extracellular starch-like compounds also occur in *Pseudozyma fusiformata* and other related species. *Candida tsukubaënsis* has been transferred to *Cryptococcus* based on the presumed presence of xylose in whole-cell hydrolyzates (Weijman and Rodrigues de Miranda 1988, Weijman et al. 1988). However, the morphological appearance of this fungus is similar to that of anamorphs of Ustilaginales. Therefore, the cell wall composition was reinvestigated, and the presence of xylose could not be confirmed (H.J. Roeijmans, unpublished results). Moreover, analysis of partial nucleotide sequences of 26S ribosomal DNA strongly supports a close phylogenetic relationship with *Ustilago maydis* (Boekhout et al. 1995a).

103.8. *Ustilago maydis* (De Candolle) Corda (1842)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are fusiform, variable in size, (10–40)×(1.5–3.0) µm, with many oil droplets and polar conidiogenesis. Hyphae occur, with the cytoplasm retracted in cells, measure (20–40)×(2.0–3.0) µm, and have sterigmata on which fusiform blastoconidia are formed. Colonies are dimorphic, with the center flat, slightly pustulate to venose, dull, pale yellowish-brown to olivaceous yellow-brown, finely pruinose, but locally smooth and somewhat shiny, zonate towards the margin, radially fibrillar, and with the margin fringed.

Growth on the surface of assimilation media (glucose): Floating flocks and a sediment are formed.

Dalmau plate culture on morphology agar: After 5 days at 17°C, hyphae occur, with retracted cytoplasm, retraction septa and sterigmata on which fusiform to cylindrical blastoconidia measuring (9.0–20.0)×(2.0–3.0) µm are formed. Hyphae may disarticulate through dissolution of the cell wall between the cytoplasm-containing cells. Aerobic growth is grayish-white to gray, dull, finely velutinous, with the center raised, irregular to somewhat pustulate, and the margin fringed. The aerial mycelium consists of ramifying and acropetal chains of fusiform conidia.

Fermentation: absent.

Assimilation (25°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	s	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
Saccharate	–	Nitrite	+
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose	–	Growth at 37°C	s
Starch formation	–		

Co-Q: 10 (Boekhout et al. 1992b).
Mol% G+C: Not determined.
Cell wall hydrolyzates: Glucose, galactose, mannose and arabinose (Dörfler 1990).

Origin of the strain studied: CBS 504.76, from maize (*Zea mays*), Netherlands.
Type strain: None designated.

Comments: The description of the asexual state of *Ustilago maydis* is presented here for comparative purposes due to the close relationship between *U. maydis* (and probably other smuts occurring on monocoteledons) and the *Pseudozyma* species determined from phenotypic and molecular data.

Discussion on anamorphs of Ustilaginales: Species of *Pseudozyma,* which share morphological, physiological and biochemical characteristics, have been placed in various genera of fungi. Although there may be some disagreement regarding the introduction of anamorph names for holomorphic fungi, such as most species of the Ustilaginales, the frequent isolation of anamorphs that lack teleomorphs, requires a form-genus to tentatively categorize these anamorphs. Kurtzman (1990a) suggested that *Vanrija* R.T. Moore may be an appropriate genus for one of these anamorphs (viz. *Sporobolomyces antarcticus*). We do not favor this solution, as the genus *Vanrija* is poorly defined as a basidiomycetous counterpart of *Candida* and *Torulopsis* (Moore 1980). Recent molecular work has demonstrated that this genus is highly polyphyletic (Fell et al. 1992, 1995), and that the type species *Vanrija humicola* (Daszewska) R.T. Moore belongs to a different evolutionary lineage when compared with the anamorphs of Ustilaginales.

Pseudozyma is physiologically characterized by the

ability to assimilate *myo*-inositol and D-glucuronate, and the inability to form extracellular starch-like compounds. Morphologically, the strains usually have flat, rapidly expanding colonies, which frequently become zonate. The aerial mycelium consists of ramifying, acropetal chains of fusiform conidia; chlamydospores may be present, but ballistoconidia are absent. The available molecular data suggest that all these fungi belong to the Ustilaginales (Boekhout et al. 1995a), as has been suggested earlier based on comparative morphology (Boekhout 1987).

104. *Reniforma* Pore & Sorenson

W.G. Sorenson and R.S. Pore

Diagnosis of the genus

Asexual reproduction is by budding on a narrow base. Cells are kidney-shaped with a flat base circumscribed by a brim. The cells tend to be arranged in orderly stacks.

Sexual reproduction is unknown.

Sugars are not fermented. Neither nitrate nor *myo*-inositol is assimilated. Starch-like compounds are not produced. Diazonium blue B and urease reactions are positive.

Type species

Reniforma strues Pore & Sorenson

Species accepted

1. *Reniforma strues* Pore & Sorenson (1990)

Systematic discussion of the species

104.1. *Reniforma strues* Pore & Sorenson (1990)

Growth in glucose–yeast extract–peptone–water: After 3 days at 25°C, the cells are kidney-shaped, (3–6×2.3–5 µm), and occur singly, in pairs, or small clusters. Reproduction is by budding (Figs. 408, 409). The cells have a flat base and tend to be arranged in stacks or layers. There is a brim radiating from the basal margin of the cell. A sediment is present but no pellicle. Growth is similar on 5% or 15% malt extract. After one month at 25°C, a yellowish-white, dull, wrinkled, creeping pellicle with sediment is formed.

Growth on the surface of assimilation media: Thin, climbing pellicles are formed.

Growth on yeast extract–malt extract (YM) agar: Growth is rapid at 25–30°C. The colonies are waxy and spreading. On primary isolation media, the colonies are either spreading or yeastlike.

Dalmau plate culture on yeast morphology agar: After 7 days at 25°C, growth under the coverglass is sparse and thin. Aerobic growth is white to cream-colored, abundant, dry and pasty. The margin is irregular and pseudomycelium is not produced.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	+
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+/w

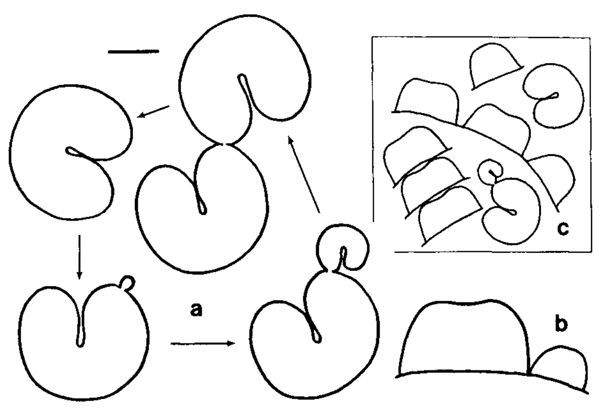


Fig. 408. *R. strues*, NRRL Y-17275. Cell morphology and budding, after 3 days in GYPW. Bar = 2 µm. (a) Budding cycle viewed from the top of the cell. (b) Side view of budding and the cell brim. (c) Typical microscopic view.

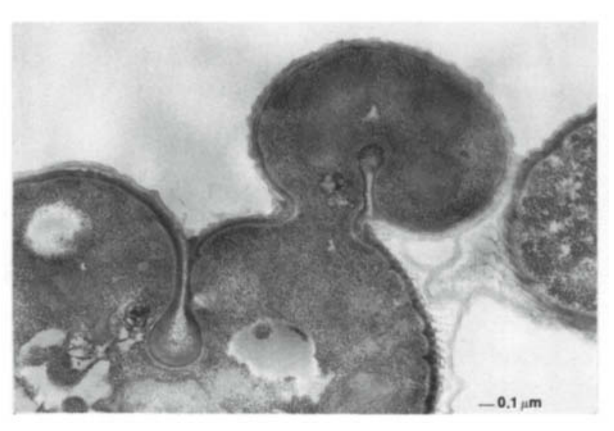


Fig. 409. *R. strues*, NRRL Y-17275. Transmission electron micrograph of the budding process. Budding may occur at other locations on the cell, but typically occurs as shown.

Additional assimilation tests and other growth characteristics:

Starch formation	–	Amylase	–
Urease	+	Growth at 37°C	–
DNAase	–		

Co-Q: Not determined.

Mol% G + C: Not determined.

Origin of the strains studied: NRRL Y-17275, and additional isolates were from a municipal wastewater treatment facility, West Virginia.

Type strain: NRRL Y-17275 (ATCC 66374, CBS 8263).

Comments on the genus

The lamellar cell wall, enteroblastic budding, and positive diazonium blue B and urease tests suggest a basidiomycetous affinity for *R. strues*. The remarkable kidney-shaped cells which produce miniature kidney-shaped buds distinguish *R. strues* from all other known yeasts.

105. *Rhodotorula* F.C. Harrison

J.W. Fell and A. Statzell-Tallman

Diagnosis of the genus

Cells are spheroidal, ovoidal or elongate. Reproduction is by multilateral or polar budding; pseudo- or true hyphae may develop. Ballistoconidia are not formed. Some strains synthesize red or yellow pigments in malt agar cultures.

Most, but not all, species lack the ability to assimilate inositol. When inositol is utilized, D-glucuronate is not assimilated. Starch-like substances are not synthesized by any of the species (see Table 85). Fermentative ability is lacking. Diazonium blue B reaction and presence of urease are positive. Coenzymes Q-9 and Q-10 are present. Xylose is lacking in cell hydrolyzates.

Some species may represent anamorphic stages of *Rhodosporidium, Leucosporidium* and other genera with simple septal pores.

Table 85
Key characteristics of the genera *Cryptococcus* and *Rhodotorula*

Starch formation	Assimilation		Tables
	Inositol	D-Glucuronate	
+	+	+	*Cryptococcus*: (1) nitrate positive, Table 76, p. 745; (2) nitrate negative, Table 77, p. 745
−	+	+	*Cryptococcus*: Table 79, p. 746
+	−	+	*Cryptococcus*: Table 78, p. 746
−	−	−	*Rhodotorula*: (1) nitrate positive, Table 90, p. 804; (2) nitrate negative, Table 91, p. 804
−	−	+	*Rhodotorula*: (1) nitrate positive, Table 88, p. 803; (2) nitrate negative, Table 89, p. 803
−	+	−	*Rhodotorula*: Table 87, p. 803

Type species

Rhodotorula glutinis (Fresenius) F.C. Harrison

Species accepted

1. *Rhodotorula acheniorum* (Buhagiar & J.A. Barnett) Rodrigues de Miranda (1975)
2. *Rhodotorula acuta* (S. Goto) Rodrigues de Miranda & Weijman (1988)
3. *Rhodotorula araucariae* Grinbergs & Yarrow (1970)
4. *Rhodotorula armeniaca* Shivas & Rodrigues de Miranda (1983)
5. *Rhodotorula aurantiaca* (Saito) Lodder (1934)
6. *Rhodotorula auriculariae* (Nakase) Rodrigues de Miranda & Weijman (1988)
7. *Rhodotorula bacarum* (Buhagiar) Rodrigues de Miranda & Weijman (1988)
8. *Rhodotorula bogoriensis* (Deinema) von Arx & Weijman (1979)
9. *Rhodotorula buffonii* (Ramírez Gómez) Roeijmans, van Eijk & Yarrow (1989)
10. *Rhodotorula diffluens* (Ruinen) von Arx & Weijman (1979)
11. *Rhodotorula ferulica* Sampaio & van Uden (1991)
12. *Rhodotorula foliorum* (Ruinen) Rodrigues de Miranda & Weijman (1988)
13. *Rhodotorula fragaria* (J.A. Barnett & Buhagiar) Rodrigues de Miranda & Weijman (1988)
14. *Rhodotorula fujisanensis* (Soneda) Johnson & Phaff (1978)
15. *Rhodotorula futronensis* (Ramírez & González) Roeijmans, van Eijk & Yarrow (1989)
16. *Rhodotorula glutinis* (Fresenius) F.C. Harrison (1928)
    a. *Rhodotorula glutinis* (Fresenius) F.C. Harrison var. *glutinis* (1958)
    b. *Rhodotorula glutinis* var. *dairenensis* Hasegawa & Banno (1958)
17. *Rhodotorula graminis* di Menna (1958)
18. *Rhodotorula hinnulea* (Shivas & Rodrigues de Miranda) Rodrigues de Miranda & Weijman (1988)
19. *Rhodotorula hordea* Rodrigues de Miranda & Weijman (1988)
20. *Rhodotorula hylophila* (van der Walt, van der Klift & D.B. Scott) Rodrigues de Miranda & Weijman (1988)
21. *Rhodotorula ingeniosa* (di Menna) von Arx & Weijman (1979)
22. *Rhodotorula javanica* (Ruinen) von Arx & Weijman (1979)

23. *Rhodotorula lactosa* Hasegawa (1959)
24. *Rhodotorula lignophila* (Dill, Ramírez & González) Roeijmans, van Eijk & Yarrow (1989)
25. *Rhodotorula marina* Phaff, Mrak & Williams (1952)
26. *Rhodotorula minuta* (Saito) F.C. Harrison (1928)
27. *Rhodotorula mucilaginosa* (Jörgensen) F.C. Harrison (1928)
28. *Rhodotorula muscorum* (di Menna) von Arx & Weijman (1979)
29. *Rhodotorula nothofagi* (Ramírez & González) Roeijmans, van Eijk & Yarrow (1989)
30. *Rhodotorula philyla* (van der Walt, van der Klift & D.B. Scott) Rodrigues de Miranda & Weijman (1988)
31. *Rhodotorula phylloplana* (Shivas & Rodrigues de Miranda) Rodrigues de Miranda & Weijman (1988)
32. *Rhodotorula pilati* (F.H. Jacob, Faure-Raynaud & Berton) J.A. Barnett, Payne & Yarrow (1983)
33. *Rhodotorula pustula* (Buhagiar) Rodrigues de Miranda & Weijman (1988)
34. *Rhodotorula sonckii* (Hopsu-Havu, Tunnela & Yarrow) Rodrigues de Miranda & Weijman (1988)

Key to species

See Table 86.

1. a Starch synthesized . . . . see key to *Cryptococcus* species: p. 743
b Starch not synthesized → 2
2(1). a Inositol assimilated → 3 (Table 87)
b Inositol not assimilated → 4
3(2). a Trehalose assimilated . . . . *R. hinnulea*: p. 816
b Trehalose not assimilated . . . . *R. phylloplana*: p. 824
4(2). a D-Glucuronate assimilated → 5
b D-Glucuronate not assimilated → 25
5(4). a Nitrate assimilated → 6 (Table 88)
b Nitrate not assimilated → 18 (Table 89)
6(5). a Raffinose assimilated → 7
b Raffinose not assimilated → 12
7(6). a Melibiose assimilated → 8
b Melibiose not assimilated → 9
8(7). a Sucrose assimilated . . . . *R. lactosa*: p. 818
b Sucrose not assimilated . . . . *R. javanica*: p. 818
9(7). a Hexadecane assimilated . . . . *R. ingeniosa*: p. 817
b Hexadecane not assimilated → 10
10(9). a L-Rhamnose assimilated . . . . *Leucosporidium scottii*: p. 673
b L-Rhamnose not assimilated → 11
11(10). a Saccharate assimilated . . . . *R. muscorum*: p. 822
b Saccharate not assimilated . . . . *R. fragaria*: p. 811
12(6). a Colony color reddish-orange . . . . *R. aurantiaca*: p. 807
b Colony color not reddish-orange → 13
13(12). a Sucrose assimilated → 14
b Sucrose not assimilated → 16
14(13). a Cellobiose assimilated → 15
b Cellobiose not assimilated . . . . *R. diffluens*: p. 810
15(14). a D-Glucosamine assimilated . . . . *R. ferulica*: p. 810
b D-Glucosamine not assimilated . . . . *R. hordea*: p. 816
16(13). a Maltose assimilated . . . . *R. buffonii*: p. 809
b Maltose not assimilated → 17
17(16). a Salicin assimilated . . . . *R. pustula*: p. 825
b Salicin not assimilated . . . . *R. foliorum*: p. 811
18(5). a Sucrose assimilated → 19
b Sucrose not assimilated → 22
19(18). a Erythritol assimilated . . . . *R. acuta*: p. 805
(also see *Sterigmatomyces elviae*): p. 844
b Erythritol not assimilated → 20
20(19). a *N*-Acetyl-D-glucosamine assimilated . . . . *R. minuta*: p. 820
b *N*-Acetyl-D-glucosamine not assimilated → 21
21(20). a Growth at 37°C . . . . *R. mucilaginosa*: p. 820
b Absence of growth at 37°C . . . . *R. marina*: p. 819
22(18). a Maltose assimilated . . . . *R. bogoriensis*: p. 808
b Maltose not assimilated → 23

Table 86
Key characteristics of species in the genus *Rhodotorula*

Species	Color[a]	Pseudo-hyphae[b]	Sta[c]	Growth[d]		Assimilation[e]																
				37°C	VF	Suc	Mal	Cel	Tre	Mel	Raf	Mlz	Rha	D-G	*N*-A	Ery	Sal	Ino	Hex	Nit	Sac	Glc
Rhodotorula acheniorum	y-p	rud	–	–	–	+	+	+	+	+	+	+	–	+	+	+	–	–	+	+	–	–
R. acuta	c	rud	–	+	w/–	+	–	+	s	–	+	–	–	–	+	+	+	–	–	–	–	+
R. araucariae	y-p	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–
R. armeniaca	o	–	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	–	–	–	–	–
R. aurantiaca	ro/y	rud	–	–	–	+	s	v	v	–	–	+	–	–	–	–	+	–	–	+	–	+
R. auriculariae	c	–	–	–	–	+	s	–	s	–	–	+	–	–	–	–	–	–	–	–	+	–
R. bacarum	b/c	rud	–	–	–	+	+	s	+	–	+	+	–	–	–	+	s	–	–	+	–	–
R. bogoriensis	c-yb	rud	–	–	–	–	+	+	+	–	–	+	–	s	+	–	s	–	–	–	w	+
R. buffonii	gb	+	–	–	–	–	+	+	+	–	–	+	–	s	s	–	s/–	–	–	+	–	+
R. diffluens	bc	+	–	–	+	+	+	–	+	–	–	+	–	+	+	+	s	–	–	+	–	+
R. ferulica	c	+	–	–	–	+	+	+	+	–	–	+	–	+	+	+	–	–	–	+	+	+
R. foliorum	y	+	–	–	–	–	–	s	+	–	–	–	–	s	+	–	–	–	–	+	–	+
R. fragaria	be	rud	–	–	+	+	+	+	+	–	+	+	–	s	+	–	+	–	–	+	–	+
R. fujisanensis	gy	rud	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–
R. futronensis	bu	rud	–	–	–	–	–	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–
R. glutinis	r/o	rud	–	+	v	+	+	v	+	–	v	+	v	–	–	–	w/+	–	+	+	–	–
R. graminis	r	v	–	–	+	+	v	v	+	–	+	–	v	–	–	–	v	–	–	+	+	–
R. hinnulea	c-bu	–	–	–	+	+	+	+	+	–	+	+	–	–	–	+	–	+	–	+	–	–
R. hordea	be	rud	–	–	–	+	+	+	+	–	–	+	–	–	–	+	+	–	+	+	–	+
R. hylophila	bc	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
R. ingeniosa	y	–	–	v	+	+	+	+	+	–	+	+	–	+	+	–	+	–	+	+	–	+
R. javanica	y	+	–	–	–	–	+	+	+	+	+	–	–	–	–	–	+	–	–	+	–	+
R. lactosa	p	–	–	–	–	+	+	+	+	+	+	+	+	–	s	–	+	–	–	+	–	+
R. lignophila	wc	–	–	–	–	–	–	+	s	–	–	–	–	–	s	–	–	–	–	–	s	+
R. marina	op	–	–	–	–	+	v	+	s	–	w	+	+	–	–	–	w	–	–	–	–	+
R. minuta	p	–	–	v	–	+	–	v	+	–	–	+	–	–	+	–	v	–	–	–	–	+
R. mucilaginosa	r	rud	–	+	v	+	v	v	+	–	+	v	v	v	–	–	v	–	–	–	–	v
R. muscorum	yc	+	–	–	s	+	+	+	+	–	+	+	–	+	+	–	+	–	–	+	+	+
R. nothofagi	y-p	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–
R. philyla	c	–	–	–	+	–	–	–	+	–	–	–	–	+	+	–	–	–	–	–	–	+
R. phylloplana	bu	–	–	–	+	+	+	–	–	–	+	+	–	–	–	+	–	s	–	+	–	–
R. pilati	c	–	–	–	–	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+	–	–
R. pustula	w-t	rud	–	–	–	–	–	+	+	–	–	–	–	+	+	–	+	–	–	+	–	+
R. sonckii	c	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–

[a] Color of culture. Abbreviations: y-p, yellowish-pink; c, cream; b/c, brown or cream; o, orange; b, brown; g, gray; be, beige; bu, buff; r, red; ro, reddish-orange; y, yellow; p, pink.

[b] Presence of pseudohyphae. Abbreviation: rud, rudimentary.

[c] Starch formation.

[d] Growth at 37°C; growth in vitamin-free medium.

[e] Abbreviations: Suc, sucrose; Mal, maltose; Cel, cellobiose; Tre, trehalose; Mel, melibiose; Raf, raffinose; Mlz, melezitose; Rha, L-rhamnose, D-G, D-glucosamine, *N*-A, *N*-acetyl-D-glucosamine; Ery, erythritol; Sal, salicin; Ino, inositol; Hex, hexadecane; Nit, nitrate, Sac, saccharate, Glc, D-glucuronate.

Table 87

Species in the genus *Rhodotorula* with the following characteristics: starch formation –, inositol +, D-glucuronate –, nitrate +

Species	Starch formation	Assimilation			
		Inositol	D-Glucuronate	Nitrate	Trehalose
Rhodotorula hinnulea	–	+	–	+	+
R. phylloplana	–	s	–	+	–

Table 88

Species in the genus *Rhodotorula* with the following characteristics: starch formation –, inositol –, D-glucuronate +, nitrate +

Species	Starch formation	Reddish colony color	Assimilation[a]												
			Ino	Glc	Nit	Suc	Mal	Cel	Mel	Raf	Rha	D-G	Sal	Hex	Sac
Rhodotorula aurantiaca	–	+	–	+	+	+	s	v	–	–	–	–	+	–	–
R. buffonii	–	–	–	+	+	–	+	+	–	–	–	s	v	–	–
R. diffluens	–	–	–	+	+	+	+	–	–	–	–	+	s	–	–
R. ferulica	–	–	–	+	+	+	+	+	–	–	–	+	–	–	+
R. foliorum	–	–	–	+	+	–	–	s	–	–	–	s	–	–	–
R. fragaria	–	–	–	+	+	+	+	+	–	+	–	s	+	–	–
R. hordea	–	–	–	+	+	+	+	+	–	–	–	–	+	+	–
R. ingeniosa	–	–	–	+	+	+	+	+	–	+	–	+	+	+	–
R. javanica	–	–	–	+	+	–	+	+	+	+	–	–	+	–	–
R. lactosa	–	+	–	+	+	+	+	+	+	+	+	–	+	–	–
R. muscorum	–	–	–	+	+	+	+	+	–	+	–	+	+	–	+
R. pustula	–	–	–	+	+	–	–	+	–	–	–	+	+	–	–
Leucosporidium scottii	–	–	–	+	+	+	+	w	–	+	+	+	+	–	v

[a] Abbreviations: Ino, inositol; Glc, D-glucuronate; Nit, nitrate; Suc, sucrose; Mal, maltose; Cel, cellobiose; Mel, melibiose; Raf, raffinose; Rha, L-rhamnose; D-G, D-glucosamine; Sal, salicin; Hex, hexadecane; Sac, saccharate.

Table 89

Species in the genus *Rhodotorula* with the following characteristics: starch formation –, inositol –, D-glucuronate +, nitrate –

Species	Starch formation	Growth at 37°C	Assimilation[a]								
			Ino	Glc	Nit	Suc	Mal	Cel	Tre	*N*-A	Ery
Rhodotorula acuta	–	+	–	+	–	+	–	+	s	+	+
R. bogoriensis	–	–	–	+	–	–	+	+	+	+	–
R. hylophila	–	–	–	+	–	–	–	–	–	–	–
R. lignophila	–	–	–	+	–	–	–	+	s	s	–
R. marina	–	–	–	+	–	+	v	+	s	–	–
R. minuta	–	v	–	+	–	+	–	v	+	+	–
R. mucilaginosa	–	+	–	v	–	+	v	v	+	–	–
R. philyla	–	–	–	+	–	–	–	–	+	+	–

[a] Abbreviations: Ino, inositol; Glc, D-glucuronate; Nit, nitrate; Suc, sucrose; Mal, maltose; Cel, cellobiose; Tre, trehalose; *N*-A, *N*-acetyl-D-glucosamine; Ery, erythritol.

23(22). a Cellobiose assimilated .... *R. lignophila*: p. 818
b Cellobiose not assimilated → 24
24(23). a Trehalose assimilated .... *R. philyla*: p. 823
b Trehalose not assimilated .... *R. hylophila*: p. 817

25(4). a Nitrate assimilated → 26 (Table 90)
b Nitrate not assimilated → 33 (Table 91)
26(25). a Sucrose assimilated → 27
b Sucrose not assimilated → 32
27(26). a Melezitose assimilated → 28
b Melezitose not assimilated → 31
28(27). a Erythritol assimilated → 29
b Erythritol not assimilated → 30
29(28). a Melibiose assimilated .... *R. acheniorum*: p. 805
b Melibiose not assimilated .... *R. bacarum*: p. 808
30(28). a Colony cream-colored .... *R. pilati*: p. 824
b Colony orange to red-colored .... *R. glutinis*: p. 814
31(27). a Growth in vitamin-free medium .... *R. graminis*: p. 815
b Absence of growth in vitamin-free medium .... see *Rhodosporidium malvinellum*: p. 686
32(26). a Growth in vitamin-free medium .... *R. araucariae*: p. 806
b Absence of growth in vitamin-free medium .... *R. sonckii*: p. 826
33(25). a Sucrose assimilated → 34
b Sucrose not assimilated → 35
34(33). a Raffinose assimilated .... *R. mucilaginosa*: p. 820
b Raffinose not assimilated .... *R. auriculariae*: p. 807
35(33). a Cellobiose assimilated → 36
b Cellobiose not assimilated .... *R. armeniaca*: p. 806
36(35). a Trehalose assimilated .... *R. futronensis*: p. 813
b Trehalose not assimilated → 37
37(36). a Growth in vitamin-free medium .... *R. nothofagi*: p. 823
b Absence of growth in vitamin-free medium .... *R. fujisanensis*: p. 812

Table 90
Species in the genus *Rhodotorula* with the following characteristics: starch formation –, inositol – D-glucuronate –, nitrate +

Species	Starch formation	Red colony color	Assimilation[a]							Growth in vitamin-free medium
			Ino	Glc	Nit	Suc	Mel	Mlz	Ery	
Rhodotorula acheniorum	–	–	–	–	+	+	+	+	+	–
R. araucariae	–	–	–	–	+	–	–	–	–	+
R. bacarum	–	–	–	–	+	+	–	+	+	–
R. glutinis	–	+	–	–	+	+	–	+	–	v
R. graminis	–	+	–	–	+	+	–	–	–	+
R. pilati	–	–	–	–	+	+	–	+	–	–
R. sonckii	–	–	–	–	+	–	–	–	–	–
Rhodosporidium malvinellum	–	–	–	–	+	+	–	–	–	–

[a] Abbreviations: Ino, inositol; Glc, D-glucuronate; Nit, nitrate; Suc, sucrose; Mel, melibiose; Mlz, melezitose; Ery, erythritol.

Table 91
Species in the genus *Rhodotorula* with the following characteristics: starch formation – inositol –, D-glucuronate –, nitrate –

Species	Starch formation	Assimilation[a]							Growth in vitamin-free medium
		Ino	Glc	Nit	Suc	Cel	Tre	Raf	
Rhodotorula armeniaca	–	–	–	–	–	–	–	–	–
R. auriculariae	–	–	–	–	+	–	s	–	–
R. fujisanensis	–	–	–	–	–	+	–	–	–
R. futronensis	–	–	–	–	–	+	+	–	–
R. mucilaginosa	–	–	v	–	+	v	+	+	v
R. nothofagi	–	–	–	–	–	+	–	–	+

[a] Abbreviations: Ino, inositol; Glc, D-glucuronate; Nit, nitrate; Suc, sucrose; Cel, cellobiose; Tre, trehalose; Raf, raffinose.

Systematic discussion of the species

105.1. *Rhodotorula acheniorum* (Buhagiar & J.A. Barnett) Rodrigues de Miranda (1975)

Synonym:
Sterigmatomyces acheniorum Buhagiar & J.A. Barnett (1973)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ellipsoidal, mostly elongate, (1.6–3.9)×(2.4–8.2) μm, and contain conspicuous fat globules. The bud may be borne on a short pedicel. After one month there is a ring and a sediment; no pellicle forms (Fig. 410).

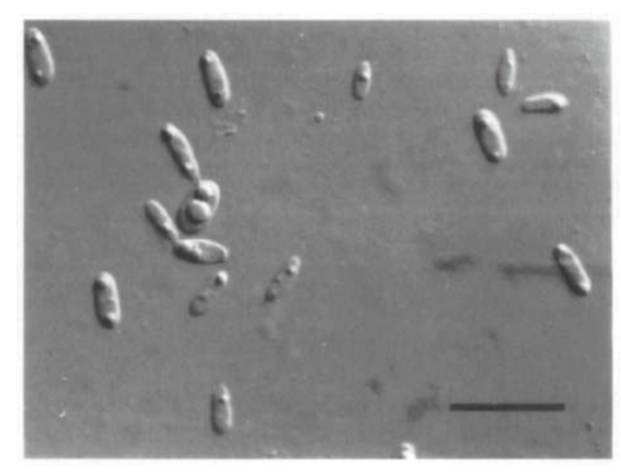


Fig. 410. *R. acheniorum,* CBS 6386. Cells grown in glucose–yeast extract–peptone water, for 3 days at 25°C, budding on short pedicels. Bar = 10 μm.

Growth on 5% malt agar: After 3 days at 25°C, the colony is buff, occasionally pale yellowish-pink, and smooth to rough. After 3 weeks or longer, the colony is pale pink-vinaceous with well-defined margins.

Dalmau plate culture on corn meal agar: After one week, short chains of budding cells with sparse pseudomycelium are present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	w
Trehalose	+	Galactitol	–
Lactose	w	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	+
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	w		

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G + C: 53.9, CBS 6386 (BD: Kurtzman, personal communication).
Cell hydrolyzates: Mannose low, malic acid (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Fresh strawberries in England (Buhagiar and Barnett 1973).
Type strain: CBS 6386.

Comments: The original isolations, from strawberries (Buhagiar and Barnett 1973), occurred in three successive years (1969, 1970 and 1971); in addition, the 1990 "CBS List of Yeasts" includes an isolate from the leaves of *Callistemon viminalis* in Australia from J.F. Brown. The original description reported that buds were formed on sterigmata, ~1 μm in length, and separation of the cell and its bud occurred at the cell, at the bud, or midway between the cells. This method of cell separation does not conform to the description in the diagnosis of the genus *Sterigmatomyces,* which is limited to mid-cell separation. Rodrigues de Miranda (1975) did not observe sterigmata and reported that the divided cells were connected by "sharp tips of both cells". Based on this mode of cell separation and the red color of the colony, Rodrigues de Miranda (1975) transferred the species from *Sterigmatomyces* to *Rhodotorula.* Nucleotide sequence analysis in our laboratory (unpublished) indicates that this species is related to the Ustilaginales. This relationship is also suggested by the low level of mannose in the cell hydrolyzates, which is characteristic of the smut-related yeasts.

105.2. *Rhodotorula acuta* (S. Goto) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonyms:
Candida acutus S. Goto (1979a)
Candida dulciaminis Tokuoka, Ishitani, S. Goto & Komagata (1987)
Rhodotorula dulciaminis (Tokuoka, Ishitani, S. Goto & Komagata) Roeijmans, van Eijk & Yarrow (1989)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to ellipsoidal, (2.7–5)×(3–8) μm, and some cells taper to a sharp point. Reproduction is by polar budding, often on a short pedicel; the cells occur singly, in pairs, chains and clusters. After one month a sediment is present; a thin pellicle or ring occurs.

Growth on 5% malt extract agar: After one month at 25°C, the colony is cream-colored, raised, the surface is dull becoming slightly rugoid, and the margin is smooth and entire.

Dalmau plate culture on corn meal agar: After one month at 19°C, a primitive pseudomycelium is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	s	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	ws
D-Xylose	+	Succinate	s
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	w/–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	10% NaCl/5% glucose	+
5-Keto-D-gluconate	n	Starch formation	–
Saccharate	–	Urease	+
D-Glucuronate	+	Gelatin liquefaction	–
50% (w/w) Glucose–yeast extract agar	+	Growth at 37°C	+

Co-Q: 9 (Goto 1979a).

Mol% G+C: 54.7 (T_m: Goto 1979a); 52.6 (HPLC: Tokuoka et al. 1987).

Cell hydrolyzates: Mannose dominant, hexitol (probably mannitol), pentitol (probably arabinitol), and tetritol (probably erythritol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Sulfited grape must of a Koshu variety grape in Japan, Oct. 1976 (Goto 1979a); sponge cake, Ibaraki Pref., Japan, May 1984 (Tokuoka strain 155-CA).

Type strain: CBS 7053 (Goto strain C 96-2).

Comments: Our assimilation results agree with the original description except for melezitose assimilation, which Goto (1979a) described as positive. Morphology, assimilation patterns and LSU rDNA sequence analysis indicate that *R. dulciaminis* is a synonym of *R. acuta.* These same criteria indicate that *R. acuta* is a synonym of *Sterigmatomyces elviae.*

105.3. *Rhodotorula araucariae* Grinbergs & Yarrow (1970b)

Growth in 5% malt extract: After 3 days at 24°C, the cells are ovoidal to subglobose, (3.0–6.0)×(3.4–7.4) µm, single or budding. Grinbergs and Yarrow (1970b) report cell lengths to 11 µm. After one month there is a ring and moderate sediment; there is no pellicle.

Growth on 5% malt agar: After 3 days at 24°C, the colony is pale yellowish-pink, smooth, and glistening. The cross section is raised, the texture soft, the margin entire. After one month the color is more intense.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	w
D-Xylose	+	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Sugiyama et al. 1985).

Mol% G+C: 64.6 (T_m: Sugiyama et al. 1985).

Cell hydrolyzates: Fucose and mannose dominant; hexitol (probably mannitol) and pentitol (probably arabinitol) present (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Rotting conifer, *Araucaria araucana,* in Llaima, Chile, in December 1968 (Grinbergs and Yarrow 1970b).

Type strain: CBS 6031 (Grinbergs strain number 1922).

Comments: Based on nucleotide sequence alignments of a region of the LSU rRNA (Fell et al. 1992), *R. araucariae* is closely related to *Rhodosporidium kratochvilovae.*

105.4. *Rhodotorula armeniaca* Shivas & Rodrigues de Miranda (1983b)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoid to elongate, measuring (2–6.7)×(4–9.4) µm, and occur singly or in pairs. After one month, a light sediment is present.

Growth on 5% malt extract agar: After one month at 19°C, the streak colony is orange, dull or semi-glistening, and butyrous with undulated margins.

Dalmau plate culture on corn meal agar: After one week at 19°C, pseudomycelium is not formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	–
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	w/–
Trehalose	–	Galactitol	w/–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	w
L-Arabinose	+	Citrate	–
D-Arabinose	s	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: Not determined.
Cell hydrolyzates: Not determined.

Origin of the strain studied: One strain of this species was isolated in 1981 from the surface of leaves of the bottle brush, *Callistemon viminalis* (Soland ex Gaertr.) G. Don ex Loud., growing at Armidale, New South Wales, Australia.

Type strain: CBS 8076.

Comments: To ensure survival, this strain must be transferred biweekly; otherwise, we recommend storage at –80°C or in the vapor phase of liquid nitrogen.

105.5. *Rhodotorula aurantiaca* (Saito) Lodder (1934)

Synonyms:
Torula aurantiaca Saito (1922)
Torulopsis aurantiaca (Saito) Ciferri & Redaelli (1925)
Chromotorula aurantiaca (Saito) F.C. Harrison (1928)
Rhodotorula glutinis (Fresenius) F.C. Harrison var. *aurantiaca* (Saito) Hasegawa (1958)
Mycotorula colostri Castelli (1932)
Rhodotorula colostri (Castelli) Lodder (1934)
Rhodotorula crocea Shifrine & Phaff (1956)

Growth in 5% malt extract: After 3 days at 19°C, the cells are elongate ovoidal to cylindrical, (3–5)×(6–13) μm, occasionally 16 μm or longer; in pairs or single, sometimes in short chains; little sediment, but there may be a thin, partial ring. After one month, a thin to well-developed orange or red ring and a moderate amount of sediment are present.

Growth on 5% malt agar: The cell morphology is similar to that in malt extract, but cells may be slightly longer. The streak culture after one month is reddish-orange, or dark yellow, finely wrinkled or smooth, semiglossy, soft, flat to low convex, with an entire to slightly irregular border.

Dalmau plate culture on corn meal agar: Pseudomycelium absent or rudimentary, consisting of short chains of elongate cells.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	s
Maltose	s	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	v	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	v	Gelatin liquefaction	–
Saccharate	–	PABA-free	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 25°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	–		
Starch formation	–		

Co-Q: 10 (Yamada and Kondo 1973).
Mol% G+C: 55.4, type strain (T_m: von Arx and Weijman 1979); strain *R. crocea*: 58.8 (T_m: Nakase and Komagata 1971c).
Cell hydrolyzates: Mannose dominates, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Air in Japan.

Type strain: CBS 317, isolated from atmosphere by Saito (1922), was designated as the type strain by Lodder and Kreger-van Rij (1952).

Comments: Other strains have been isolated from Japan, Europe, North America, the Antarctic, Atlantic and Indian Oceans. Sources include: clinical (1), unknown (1), bark beetle (1), plants and soils (7), marine waters (10).

105.6. *Rhodotorula auriculariae* (Nakase) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonyms:
Torulopsis auriculariae Nakase (1971a)
Candida auriculariae (Nakase) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are spherical to ovoidal,

(3.4–8)×(4.7–11.4) μm, and occur singly, in pairs or in clusters. A sediment is formed. After one month a ring is present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is cream-colored, mucoid, glistening, moist, convex and entire.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	w
Sucrose	+	Glycerol	–
Maltose	s	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 54.4 (T_m: Nakase 1971a).
Cell hydrolyzates: Fucose, rhamnose, mannose dominant, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Fruiting body of *Auricularia auricula-judae,* in Zaruga-take (Mt. Zaru), Hamanashi Prefecture, Japan, May 1965 (strain AJ 4404, IFO 1580).

Type strain: CBS 6379.

105.7. *Rhodotorula bacarum* (Buhagiar) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonyms:
Torulopsis bacarum Buhagiar (1975)
Candida bacarum (Buhagiar) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Growth in 5% malt extract: After 4 days at 25°C, the cells are ovoidal and elongate, (2.5–4)×(5.5–10) μm. After one month a sediment and light ring are present.

Growth on 5% malt extract agar: After one month at 25°C, the streak culture is brownish or cream-colored, smooth, convex, with an entire to slightly undulating margin.

Dalmau plate culture on corn meal agar: After one week at 25°C, the pseudomycelium is poorly developed, consisting of ramified chains of ovoid cells.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	s	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	s
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	s
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 52.4 (T_m: Meyer et al. 1984).
Cell hydrolyzates: Mannose low, hexitol (probably mannitol), malic acid (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Black currants, raspberries (*Rubus idaeus* cultivar Malling Jewel) and strawberries (*Fragaria ananassa* cultivar Cambridge Favourite).

Type strain: CBS 6526, isolated from black currants (*Ribes nigrum* cultivar Baldwin) by Buhagiar (1975).

Comments: Other strains have been isolated from dolphin (mammal) skin near the Netherlands by M. Luykx (CBS 7102) and air in Portugal by N. van Uden (CBS 7415). Nucleotide sequence analysis (unpublished) in our laboratory indicates that this species is related to the Ustilaginales. This relationship is also suggested by the low level of mannose in the cell hydrolyzates, which is a characteristic of the smut-like yeasts.

105.8. *Rhodotorula bogoriensis* (Deinema) von Arx & Weijman (1979)

Synonyms:
Candida bogoriensis Deinema (1961)
Candida bogoriensis Deinema var. *lipolytica* Ruinen (1963)
Vanrija bogoriensis (Deinema) R.T. Moore (1980)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal-elongate, (2.0–5)×(5–17) μm, and occur singly or in pairs. After one month, a thin ring and heavy sediment have formed.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is cream to yellowish-brown, mucoid, soft and smooth.

Dalmau plate culture on corn meal agar: After one week at 25°C, there are branched chains of short pseudohyphae present.

Fermentation: absent.

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	+
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	–	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	s
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+/w
L-Arabinose	+	Citrate	+/w
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	w	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 25°C	+
10% NaCl/5% glucose	+	Growth at 30°C	–

Co-Q: 10 (Yamada and Kondo 1972a).
Mol% G+C: 57.3, type strain (T_m: Meyer and Phaff 1972).
Cell hydrolyzates: Fucose, rhamnose, mannose dominant (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Surface of leaves of the flowering shrub *Randia malleifera* in Bogor, Indonesia.

Type strain: CBS 4101.

Comments: The original description reports that growth in ethanol was negative, which differs from our results.

105.9. *Rhodotorula buffonii* (Ramírez Gómez) Roeijmans, van Eijk & Yarrow (1989)

Synonyms:
Torulopsis buffonii Ramírez Gómez (1957)
Paratorulopsis buffonii (Ramírez Gómez) Novák & Zsolt (1961)
Candida buffonii (Ramírez Gómez) van Uden & H.R. Buckley (1970)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are elongate, (2–3.5)×(10–22) μm. After one month, a ring, pellicle, and sediment have formed.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is grayish-brown, glistening, soft and smooth. On potato agar the streak culture is mucoid.

Dalmau plate culture on corn meal agar: Pseudomycelium is abundantly formed, consisting of branched chains of elongate cells.

Fermentation: absent.

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	s
Galactose	–	Methanol	–
L-Sorbose	s	Ethanol	s
Sucrose	–	Glycerol	s
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	–
D-Xylose	s	Succinate	v
L-Arabinose	s	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Gelatin liquefaction	–
5-Keto-D-gluconate	n	Biotin-free	–
Saccharate	–	Inositol-free	–
D-Glucuronate	+	Niacin-free	–
50% (w/w) Glucose–yeast extract agar	–	Thiamine-free	–
		Growth at 25°C	+
10% NaCl/5% glucose	s	Growth at 30°C	–
Starch formation	–		
Urease	+		

Co-Q: Not determined.
Mol% G+C: 50.8, type strain (T_m: Meyer et al. 1984).
Cell hydrolyzates: Xylose absent, fucose and rhamnose present (Roeijmans et al. 1989).

Origin of the strain studied: Carpophore of *Boletus edulis* var. *alba* (Ramírez Gómez 1957) (1).

Type strain: ATCC 18813.

Comments: The type strain of *R. buffonii* was listed in van Uden and Buckley (1970), Barnett et al. (1983) and Meyer et al. (1984) as CBS 2838. Van Uden and Buckley (1970) and Meyer et al. (1984) depict elongate cells for this strain. In contrast, a photograph of CBS 2838 in Barnett et al. (1983) illustrates ovoid cells. Additionally, Barnett et al. (1983) reported that this strain is diazonium blue B negative, an atypical reaction for a basidiomycete. Barnett et al. (1990) list the type strain as CBS 7150 with elongate cells and a diazonium blue B positive reaction. According to Yarrow (personal communication) CBS 2838 was lost and CBS 7150, a subculture of CBS 2838, was substituted as the type strain. To add to the confusion, there are two strains of CBS 7150 in

circulation. One strain is diazonium blue B negative and the cells are ovoid, similar to the cell photograph of CBS 2838 in Barnett et al. (1983). Based on the diazonium blue B reaction and nucleotide sequence alignment data (Fell et al. 1992), that strain is an ascomycete. The other strain is probably an authentic strain of *Candida buffonii*; the diazonium blue B reaction is positive and the cells are elongate, similar to those in van Uden and Buckley (1970).

Because of the questionable identity of the type strain, we examined strain ATCC 18813, which ATCC lists as CBS 2838. We found that ATCC 18813 cells resemble the elongate cell descriptions of *Candida buffonii* and the diazonium blue B reaction is positive, which clarifies its position as a basidiomycete. Therefore, we selected ATCC 18813 to be the type strain, because it appears to represent the original CBS 2838. The standard description presented here (with the exceptions of the mol% G + C and cell hydrolyzates) is taken from observations and tests on ATCC 18813.

105.10. *Rhodotorula diffluens* (Ruinen) von Arx & Weijman (1979)

Synonyms:
Candida diffluens Ruinen (1963)
Vanrija diffluens (Ruinen) R.T. Moore (1980)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are subglobose, ovoid, or slightly irregular. (3–5)×(5–11) μm. The cells are encapsulated.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is brownish cream-colored, glistening, soft, smooth, and mucoid.

Dalmau plate culture on corn meal agar: The pseudomycelium is either primitive, consisting of branched chains of cylindrical cells, or displays long pseudohyphal cells bearing spindle-shaped blastospores (Fig. 411).

Fermentation: absent.

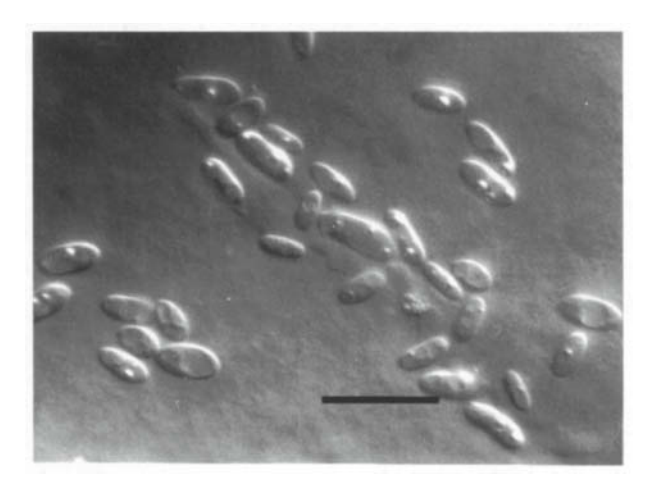


Fig. 411. *R. diffluens*, CBS 5233. Cells grown on corn meal agar for 5 days at 25°C. Bar = 10 μm.

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	+
Galactose	s	Methanol	−
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	s
Maltose	+	Erythritol	+
Cellobiose	−	Ribitol	+
Trehalose	+	Galactitol	−
Lactose	−	D-Mannitol	+
Melibiose	−	D-Glucitol	+
Raffinose	−	α-Methyl-D-glucoside	−
Melezitose	+	Salicin	s
Inulin	−	D-Gluconate	s
Soluble starch	−	DL-Lactate	−
D-Xylose	v	Succinate	+
L-Arabinose	−	Citrate	s
D-Arabinose	−	Inositol	−
D-Ribose	−	Hexadecane	−
L-Rhamnose	−	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	−
5-Keto-D-gluconate	n	Urease	+
Saccharate	−	Gelatin liquefaction	−
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	−	Growth at 37°C	−
10% NaCl/5% glucose	−		

Co-Q: 10 (Yamada and Kondo 1972a).
Mol% G + C: 60.5 (T_m: Nakase and Komagata 1971c).
Cell hydrolyzates: Fucose, rhamnose, mannose dominate, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Leaf of *Tillandsia usneoides* (bromeliad) in Dutch Guyana (Ruinen 1963).
Type strain: CBS 5233.

105.11. *Rhodotorula ferulica* Sampaio & van Uden (1991)

Growth in 5% malt extract: After 3 days at 19°C, the cells are ovoidal to spherical, encapsulated, measuring (2.7–4.7)×(4.0–6.7) μm, and occur singly or in pairs. Buds may form on a short neck. After one month, there is a light sediment, but no ring or pellicle.

Growth on 5% malt extract agar: After one month at 19°C, the streak colony is cream-colored, mucoid, raised, glistening and the margin is entire.

Dalmau plate culture on corn meal agar: After one month at 19°C, both true and pseudomycelium are formed. Blastoconidia form laterally on the hyphal cells.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	s	Methanol	–
L-Sorbose	s	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	ws
D-Xylose	–	Succinate	s
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Vannilic acid	+
5-Keto-D-gluconate	n	Veratric acid	+
Saccharate	+	Ferulic acid	+
D-Glucuronate	+	*p*-Hydroxybenzoic acid	+
Arbutin	+	*m*-Hydroxybenzoic acid	+
Cadaverine	v	Protocatechuic acid	+
Creatine	v	*p*-Coumaric acid	+
Creatinine	–	Caffeic acid	+
L-Lysine	+	Cinnamic acid	–
Ethylamine hydrochloride	v	Syringic acid	–
50% (w/w) Glucose–yeast extract agar	–	Sinapic acid	–
		Gallic acid	–
10% NaCl/5% glucose	–	Salicylic acid	–
Starch formation	–	Gentistic acid	–
Urease	+	Guaiacol	–
Gelatin liquefaction	–	Sodium nitrate	+
Glucono-δ-lactone	+	0.01% Cycloheximide	+
L-Tartaric acid	+	0.1% Cycloheximide	–
Malic acid	v	Growth at 25°C	+
Vanillyl alcohol	+	Growth at 30°C	–
Veratryl alcohol	+		

Co-Q: 10 (Sampaio and van Uden 1991).
Mol% G + C: 66.1 (T_m: Sampaio and van Uden 1991).
Cell hydrolyzates: Xylose absent (Sampaio and van Uden 1991).

Origin of the strains studied: Three strains were isolated about one mile upstream from the mouth of the Tagus River, Oeiras, Portugal. The main source of pollution in this river is a tobacco processing plant (Sampaio and van Uden 1991).

Type strain: CBS 7416 (IGC 4524).

Comments: The original description noted growth at 30°C, which we were not able to confirm.

105.12. *Rhodotorula foliorum* (Ruinen) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonym:
Candida foliarum Ruinen (1963)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are slender, ovoid-elongate to cylindrical, (1.5–4) × (4–12) μm. They may be single or in pairs and are encapsulated. A ring may be present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is yellowish, glistening, mucoid, smooth and entire.

Dalmau plate culture on corn meal agar: The abundant pseudomycelium is primitive, consisting of ramified chains of cylindrical cells.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	s	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	s	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	s
D-Xylose	s	Succinate	s
L-Arabinose	v	Citrate	s
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	s	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Urease	+
5-Keto-D-gluconate	n	Gelatin liquefaction	–
Saccharate	–	Biotin-free	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 25°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	–		
Starch formation	–		

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G + C: 56.0 (T_m: G.A. Poot, personal communication, in Barnett et al. 1990).
Cell hydrolyzates: Fucose, rhamnose, mannose dominate, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Leaves of *Drymoglossum pilloselloides* growing epiphytically in the Botanical Garden, Bogor, Indonesia (Ruinen 1963).

Type strain: CBS 5234.

Comments: The original specific epithet was incorrectly spelled *foliarum* and was changed by Yarrow (Barnett et al. 1983). According to the original description lactose was not assimilated, which differs from our results.

105.13. *Rhodotorula fragaria* (J.A. Barnett & Buhagiar) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonyms:
Torulopsis fragaria J.A. Barnett & Buhagiar (1971)
Candida fragariorum (J.A. Barnett & Buhagiar) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal or cylindrical. In defined medium with glucose as the sole carbon source, the cells measure (1.8–6.1)×(3.4–12.4) µm.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is beige, smooth, shiny and flat with an entire or slightly irregular edge.

Dalmau plate culture on corn meal agar: Short chains of cells are present after one week.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	s	Citrate	+
D-Arabinose	s	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	s	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G + C: 56.6 (T_m: G.A. Poot, personal communication, in Barnett et al. 1990).

Cell hydrolyzates: Fucose, mannose dominate, hexitol (probably mannitol) and pentitol (probably arabinitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Strawberries (CBS 6254).

Type strain: CBS 6254.

Comments: Thirty-five strains were isolated from fresh strawberries and five strains from black currants in the United Kingdom (Barnett and Buhagiar 1971). Based on standard carbon and nitrogen utilization tests, this species resembles *R. muscorum.* Molecular sequence alignment data confirms that the two species are closely related (unpublished data, Fell et al.). Taxonomically the two species can be separated on the following: *R. fragaria* assimilates L-arabinose slowly, *R. muscorum* does not. *R. muscorum* assimilates saccharate and slowly assimilates galactitol, *R. fragaria* does not assimilate either compound.

105.14. *Rhodotorula fujisanensis* (Soneda) Johnson & Phaff (1978)

Synonyms:

Torulopsis fujisanensis Soneda (1959)
Candida fujisanensis (Soneda) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Vanrija fujisanensis (Soneda) R.T. Moore (1980)

Growth in glucose–yeast extract–peptone water: After 3 days at 19°C, the cells are long-ovoidal to cylindrical, (2.5–5)×(7–15) µm. A ring and an incomplete pellicle are present.

Growth on glucose–yeast extract–peptone agar: After one month at 19°C, the streak culture is grayish-yellow, semi-dull, soft, smooth or finely punctate.

Dalmau plate culture on corn meal agar: A rudimentary pseudomycelium is formed that consists of ramified chains of ovoid cells.

Life cycle: Mixing of cells from strains CBS 6371 and CBS 4551 on potato dextrose agar, followed by incubation at 12–18°C for 18 days can result in the formation of hyphae with clamps (Fig. 412) and teliospores. The teliospores (Fig. 413) are globose, 10–26 µm diameter, to occasionally elongate. The formation of teliospores is inconsistent and attempts to induce germination have been unsuccessful.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	–	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	–
L-Arabinose	+	Citrate	w
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	s	Growth at 30°C	–
10% NaCl/5% glucose	+		

Co-Q: 9 (Goto and Oguri 1983).

Mol% G + C: 63.4 (T_m: Goto and Oguri 1983).

Cell hydrolyzates: Fucose, rhamnose, mannose dominate, hexitol (probably mannitol), citric acid (Weijman and Rodrigues de Miranda 1988).

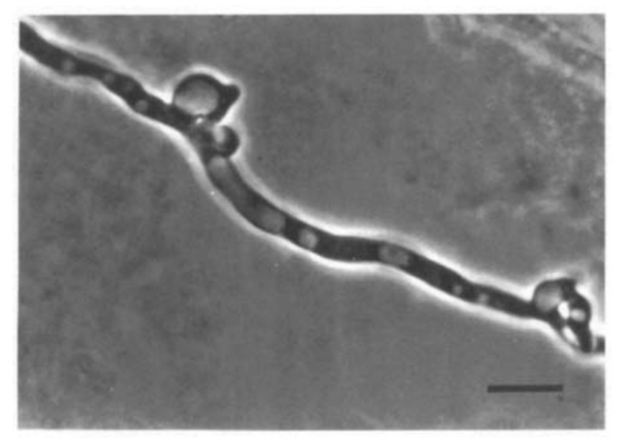


Fig. 412. *R. fujisanensis*, CBS 4551×CBS 6371. Cells of opposite mating types were mixed on potato dextrose agar and incubated at 12°C. After 8 days, hyphae with clamps and teliospores formed. Bar = 10 µm.

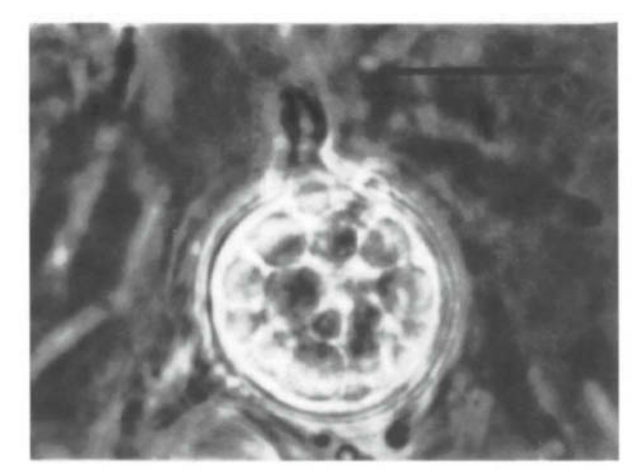


Fig. 413. *R. fujisanensis*, CBS 4551×CBS 6371. Cells of opposite mating types were mixed on potato dextrose agar and incubated at 12°C. Teliospore formed after 8 days. Bar = 10 µm.

Origin of the strains studied: Hare feces, Japan (1); leaves, France, by M.R. Bardiz (1).

Complementary mating types: CBS 4551, from feces of a hare in Japan, NI 7646, MT *A1* (Soneda 1959), and CBS 6371, from leaves in France, MT *A2*.

Type strain: CBS 4551.

Comments: The species was originally described in the genus *Torulopsis*. Subsequently, Johnson and Phaff (1978) chemically extracted and characterized carotenoids from this species and transferred the species to *Rhodotorula*. The formation of sexually produced teliospores demonstrates that *R. fujisanensis* belongs in a teleomorphic genus. Generic placement and formal description has been delayed due to an inability to induce germination of the teliospores and complete the details of the life cycle. Nucleotide sequence analysis in our laboratory (unpublished) indicates that *R. fujisanensis* is a member of the Sporidiales with a closer affinity to *Leucosporidium* than to *Rhodosporidium*.

Several other strains have been isolated: frass of *Ruguloscolytus rugulosus*, M.C. Pignal (1); wild grapes (*Vitis coignetiae*) mating type *A1*, CBS 8056, by Goto, Japan; leaves of the California laurel (*Umbellularia californica*), rotting *Opuntia* cactus tissue in Australia and fermenting soap berries (*Sapindus saponaria*) from Hawaii.

105.15. *Rhodotorula futronensis* (Ramírez & González) Roeijmans, van Eijk & Yarrow (1989)

Synonym:

Apiotrichum futronensis Ramírez & González (1984d)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal to elongate, measuring (2–5.4)×(3.4–9.4) µm. The cells reproduce by budding and occur singly, or in clusters. After one month, a ring and a heavy sediment are present.

Growth on 5% malt extract agar: After one month at 25°C, the colony is buff colored, flat in cross section, butyrous, the surface is dull, the margin is entire and fringed with pseudomycelium.

Dalmau plate culture on corn meal agar: After one month at 19°C, short pseudohyphae are present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	w
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G + C: Not determined.

Cell hydrolyzates: Xylose absent, fucose and rhamnose present (Roeijmans et al. 1989).

Origin of the strain studied: Rotting wood of a laurel, *Laurelia sempervirens*, in the evergreen rainy Valdivian forest near Futrono, Chile.

Type strain: CBS 8163 (IJFM 6008), from rotting wood of *Laurelia sempervirens*.

Comments: A strain from the intestinal tract of a fly, *Scaptomyza multispinosa*, collected in Chile by Ramírez and González (1984d) was not included in this study.

105.16. *Rhodotorula glutinis* (Fresenius) F.C. Harrison (1928)

This species has two varieties:

Rhodotorula glutinis (Fresenius) F.C. Harrison var. *glutinis* (1958)

Synonyms:

Blastodendrion aerius Ciferri & Redaelli (1925)
Cryptococcus glutinis Fresenius (1850)
Saccharomyces glutinis (Fresenius) Cohn (1872)
Torula glutinis (Fresenius) Pringsheim & Bilewsky (1911)
Torulopsis glutinis (Fresenius) Dodge (1935)
Saccharomyces roseus Engel (1877)
Torulopsis roseus Engel (1877)
Saccharomyces fresenii Schroeter (1908)
Mycotorula rosea-corallina Scaramella (1928)
Torula rufula Saito (1922)
Torulopsis rufula (Saito) Ciferri & Redaelli (1925)
Rhodotorula rufula (Saito) F.C. Harrison (1928)
Rhodotorula glutinis (Fresenius) F.C. Harrison var. *rufula* (Saito) Lodder (1934)
Torulopsis bronchialis Ciferri & Redaelli (1925)
Rhodotorula bronchialis (Ciferri & Redaelli) Lodder (1934)
Cryptococcus bronchialis (Ciferri & Redaelli) Nannizzi (1934)
Torulopsis saitoi Ciferri & Redaelli (1925)
Rhodotorula glutinis (Fresenius) F.C. Harrison var. *saitoi* (Ciferri & Redaelli) Lodder (1934)
Torula miniata Okunuki (1931)
Torula suganii Okunuki (1931)
Torulopsis minuta (Saito) Ciferri & Redaelli var. *americana* Ciferri (1931a)
Rhodotorula suganii (Okunuki) Lodder (1934)
Rhodotorula glutinis (Fresenius) F.C. Harrison var. *lusitanica* Marcilla, Feduchy & Gomes (1945)
Rhodotorula terrea Sugiyama & S. Goto (1969)

Rhodotorula glutinis var. dairenensis Hasegawa & Banno (1958)

Synonyms:

Torula rubra Schimon (1911)
Torula rubra Schimon var. α Saito (1922)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to globose, (2.3–5.0)×(4.0–10) μm, or more elongate, (12–16 μm), and wider (7 μm). There is usually a thin ring and a light sediment. After one month a medium to heavy, pink to orange or salmon-colored ring and a heavy sediment are present.

Growth on 5% malt agar: After one month at 25°C, the cell morphology is similar to that in malt extract, except that the cells may be longer. The streak culture is coral-red to salmon or slightly orange. The surface varies from smooth, often with fine transverse striations, to wrinkled; the appearance is from highly glossy to semiglossy. The texture varies from mucoid to pasty to slightly tough. The cross section is flat to broad convex, and the border is irregular to entire, often with rudimentary pseudomycelium.

Dalmau plate culture on corn meal agar: Pseudomycelium is usually absent or rudimentary; in some strains well-developed pseudohyphae or true hyphae with large, dark pigmented teliospores are present, which indicates self-sporulating strains of *Rhodosporidium*.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	v	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+/w
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	v	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	+
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Starch formation	–
5-Keto-D-gluconate	–	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Thiamine-free	v
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	+
10% NaCl/5% glucose	w		

Co-Q: 10 (Nakase and Komagata 1971a).

Mol% G+C: 60.0–61.2; 66.8–67.8 (T_m: Nakase and Komagata 1971a).

Cell hydrolyzates: Fucose, mannose dominate, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Supplementary description of *R. glutinis* var. *dairenensis*: The variety is similar to *R. glutinis* var. *glutinis* except that nitrate is assimilated weakly, 2-keto-D-gluconate is not assimilated and thiamine is required for growth. The nucleotide sequence analysis indicates that the variety *dairenensis* has a closer phylogenetic relationship to *R. mucilaginosa* (Fell et al. 1992) than to the variety *glutinis*.

Origin of the strains belonging to the variety *glutinis*: 370 strains from Japan, Sweden, U.S.A., Kenya, Germany, Spain, Canada, and the Atlantic, Indian and Antarctic Oceans. Sources included: air (6), human patient (1), wood pulp (20), brewery water (1), plant leaves (2), boric acid (1), brined pickles (1), olive brine (2), textiles (1), flowers (6), tree slime fluxes (4), marine (316), oil drillings (1), lake trout intestine (1),

brine shrimp (2), polluted water, sputum, unknown sources (4).

Type strain: CBS 20, isolated by Pringsheim (Berlin) from the atmosphere and sent to CBS around the year 1912.

Origin of the strain belonging to the variety *dairenensis*: The strain (CBS 4406) was isolated from the atmosphere by Saito (1922).

Type strain: CBS 4406.

Comments: *R. glutinis* var. *glutinis*, the type species of the genus, is world-wide in distribution, has been isolated from a wide variety of substrates and is probably the most prevalent species in the genus. Although some strains grow at 37°C, the species is not considered to be a human pathogen; it has been reported from skin diseases of chickens (Kwon-Chung and Bennett 1992). As indicated by the variability in taxonomic characteristics and the range of mol% G+C values, the species is polytypic, consisting of several sexually isolated species. To date, five species of *Rhodosporidium* (*R. babjevae*, *R. diobovatum*, *R. kratochvilovae*, *R. sphaerocarpum* and *R. toruloides*) have been described with phenotypic characteristics that would classify their anamorphic states as *Rhodotorula glutinis*. However, the type strain of *R. glutinis* does not mate with any of the sexual species. Based on our unpublished nucleotide sequence analyses of a partial region of the LSU rDNA, *R. glutinis* var. *glutinis* is a genetically distinct species that is more closely related to *Rhodosporidium babjevae* and *R. diobovatum* than the other three teleomorphic species. There is also a close relationship of *R. glutinis* var. *glutinis* and *R. graminis* (see *R. graminis* discussion). The variety *dairenensis* is more closely related to *R. mucilaginosa* than to the variety *glutinis*, consequently the variety *dairenensis* will probably be elevated to the rank of species when our study of the molecular systematics of the genus *Rhodotorula* is completed.

105.17. *Rhodotorula graminis* di Menna (1958a)

Synonyms:

Pichia rosa Nishiwaki (1910)

Rhodotorula rosa (Nishiwaki) S. Goto & Yokotsuka (1962a)

Growth in 5% malt extract: After 3 days at 25°C, the cells are globose to ovoidal or elongate, (2.5–7)×(4–15) μm, often having oil droplet inclusions and may occur singly, in pairs or in short chains or clusters. A thin ring and a light sediment are present. After one month a moderate to heavy pink ring, usually a heavy sediment, and occasionally moist islets may be present.

Growth on 5% malt agar: The cell morphology is similar to that in malt extract. After one month at 25°C, the streak culture is coral-red, the surface is smooth or rugose to corrugated and glossy; the texture is soft, occasionally mucoid, the cross section is flat to low convex and the margin is irregular with some rudimentary pseudomycelium.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent or rudimentary for some strains, consisting of chains of elongate cells, while other strains have a well-developed, branched pseudomycelium growing into the agar.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	–	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+/w	Succinate	+
L-Arabinose	v	Citrate	w/–
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	+		

Co-Q: 10 (Yamada and Kondo 1973).

Mol% G+C: 67.0 (T_m: Sugiyama et al. 1985); 70.0 (BD: Storck et al. 1969).

Cell hydrolyzates: Fucose (trace), mannose dominate, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: 67 strains from New Zealand, Indonesia, North America, Japan, the Indian and Atlantic Oceans. Sources included: plants (6), marine waters (59), unknown (1), soil (1).

Type strain: CBS 2826 (di Menna strain 2K53), from pasture grass on the North Island of New Zealand (di Menna 1958a).

Comments: Di Menna (1958a) made her original isolations during summer collections on North Island, New Zealand, from the leaf surfaces of pasture grasses (*Lolium perenne*, *Anthoxanthum odoratum* and *Agrostis tenuis*). *R. graminis* appears to be closely related to *R. glutinis*; Kurtzman and Fell (1991) reported 30% nDNA relatedness between the two species. Fell et al. (1992), in a study of the nucleotide sequence alignments of a partial region of the LSU rRNA, found that the two species differed by one base position.

105.18. *Rhodotorula hinnulea* (Shivas & Rodrigues de Miranda) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonym:
Cryptococcus hinnuleus Shivas & Rodrigues de Miranda (1983a)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal and measure (1.4–4.7)×(2.7–5.4) μm. They reproduce by budding and occur singly and in pairs. After one month, a thin ring and a little sediment are present.

Growth on 5% malt extract agar: After one month at 25°C, the streak colony is cream to buff-colored, flat in cross section, with a smooth, dull surface, pasty texture and a margin that is entire.

Dalmau plate culture on corn meal agar: After one month at 19°C, pseudomycelium and true mycelium are absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	s
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	w
L-Arabinose	+	Citrate	w
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: Not determined.
Cell hydrolyzates: Mannose low (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: The surface of leaves of the honeysuckle, *Banksia collina* R. Br., growing at Armidale, New South Wales, Australia. Collections were made in 1981 (Shivas and Rodrigues de Miranda 1983a).

Type strain: CBS 8079.

Comments: This species and *R. phylloplana* are the only members of the genus that utilize inositol as a sole source of carbon. In the original description, the assimilation of α-methyl-D-glucoside was negative, soluble starch and ribose were weak, in contrast to our results. The low amount of mannose in the cell hydrolyzates and nucleotide sequence analysis in our laboratory demonstrates that *R. hinnulea* is related to the yeastlike smut fungi.

105.19. *Rhodotorula hordea* Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonym:
Candida graminis Rodrigues de Miranda & Diem (1974)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ellipsoidal and elongate, (2.7–4.7)×(6–12) μm, and occur singly or in pairs. A ring and a sediment are present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is beige, smooth, creamy to slightly mucoid with an entire margin.

Dalmau plate culture on corn meal agar: Pseudomycelium is sparse and consists of branched chains of elongated cells.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	s	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	s	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	v	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	s	Hexadecane	+
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 46.9 (Barnett et al. 1990).
Cell hydrolyzates: Fucose, mannose dominates (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Barley (*Hordei hexastichi* L.) leaves from an unspecified locale (Rodrigues de Miranda and Diem 1974).

Type strain: CBS 6403.

Comments: Since the specific epithet *Rhodotorula graminis* was in use, the name *Rhodotorula hordea* was

chosen to replace *Candida graminis* (Weijman et al. 1988).

105.20. *Rhodotorula hylophila* (van der Walt, van der Klift & D.B. Scott) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonym:
Candida hylophila van der Walt, van der Klift & D.B. Scott (1971a)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are spheroidal, ovoidal, ellipsoidal or cylindrical, (1.0–3.0)×(1.5–9.0) μm, occasionally triangular or irregular, and occur singly, in pairs, short chains and small clusters. Pseudomycelium is present. After one month a granular to floccose sediment, islets and an incomplete ring are present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is brownish-cream, moist, raised, lacy, with a fringed margin.

Dalmau plate culture on corn meal agar: The pseudomycelium consists of long slender pseudohyphae often curved and bearing few blastoconidia which are often triangular, and occur in clusters or short chains.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	s
Melibiose	–	D-Glucitol	s
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	ws
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: 48.3 (Barnett et al. 1990).
Cell hydrolyzates: Fucose and mannose dominate; pentitol (probably arabinitol), tetritol (probably erythritol) are also present (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: One strain was isolated from material from the tunnels of the beetle *Xyleborus aemulus* Woll. in a deciduous tree, *Rapanea melanophloeos* (L.) Mez., growing near Knysna, Cape Province in South Africa (van der Walt et al. 1971a).

Type strain: CBS 6226.

105.21. *Rhodotorula ingeniosa* (di Menna) von Arx & Weijman (1979)

Synonyms:
Torulopsis ingeniosa di Menna (1958b)
Candida ingeniosa (di Menna) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Vanrija ingeniosa (di Menna) R.T. Moore (1980)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal to cylindrical, single or in pairs and measure (2–3.5)×(3.5–8) μm. A thin capsule may be present. After one month, a ring is present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is yellowish, mucoid, glistening, soft and smooth.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	w	Methanol	–
L-Sorbose	s	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	+	DL-Lactate	w
D-Xylose	s	Succinate	w
L-Arabinose	–	Citrate	ws
D-Arabinose	–	Inositol	–
D-Ribose	s	Hexadecane	+
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	v
10% NaCl/5% glucose	–		

Co-Q: 10 (Yamada and Kondo 1972a).
Mol% G+C: 55.6, type strain (T_m: Nakase and Komagata 1971c).
Cell hydrolyzates: Fucose, mannose dominate, hexitol (probably mannitol), pentitol (arabinitol) malic acid (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Pasture grass leaves and in lesser frequencies from the soil beneath the grass near Hamilton, New Zealand, isolated by di Menna (1958b).

Type strain: CBS 4240, di Menna strain TG27, collected in July 1957.

105.22. *Rhodotorula javanica* (Ruinen) von Arx & Weijman (1979)

Synonym:
Candida javanica Ruinen (1963)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are long-ovoidal to spindle-shaped, (1.5–3)×(7–24) µm. The cells are encapsulated; a thin ring may be present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is yellowish, glistening, slightly mucoid, soft and almost smooth.

Dalmau plate culture on corn meal agar: The pseudomycelium consists of branched chains of spindle-shaped cells in a tree-like arrangement.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 25°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	s		

Co-Q: 9 (Sugiyama et al. 1985).
Mol% G+C: 59.0 (T_m: Stenderup et al. 1972).
Cell hydrolyzates: Fucose, rhamnose, mannose dominant; hexitol (probably mannitol) (von Arx and Weijman 1979).

Origin of the strain studied: Leaf of the flame flower, *Ixora coccinea*, growing in Botanical Garden, Java (Ruinen 1963).

Type strain: CBS 5236.

105.23. *Rhodotorula lactosa* Hasegawa (1959)

Growth in 5% malt extract: After 5 days at 20°C, the cells are single or in pairs, and short-ovoidal to long-ovoidal, (2.5–4.0)×(4.5–8.0) µm, or longer, (11–12.5) µm. There is a thin incomplete ring and a light sediment. Growth is slow. After one month, there is a thin to moderate ring and a moderate sediment.

Growth on 5% malt agar: Cells in young cultures are larger than those grown in liquid malt extract. After one month at 20°C, the streak culture is pink, smooth, highly glossy, soft but not slimy, convex and shows little spreading; the margin is entire.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	s
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	s
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	s	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	ws
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	ws
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	s
D-Arabinose	s	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	+	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	PABA-free	–
D-Glucuronate	+	Thiamine-free	w/–
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	+
		Growth at 37°C	–
10% NaCl/5% glucose	–		
Starch formation	–		

Co-Q: 9 (Yamada and Kondo 1973).
Mol% G+C: 57.3, 57.6 (T_m: Nakase and Komagata 1971d).
Cell hydrolyzates: Mannose dominates, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: From air, isolated by Mikata (2).

Type strain: CBS 5826 (IFO 1423). According to the IFO catalog this strain was collected from air by R. Mikata and designated R-13-30. Apparently the original type strain (RT-82) collected by Hasegawa, from air in a cow shed or dairy, was lost (Phaff and Ahearn 1970).

105.24. *Rhodotorula lignophila* (Dill, Ramírez & González) Roeijmans, van Eijk & Yarrow (1989)

Synonym:
Candida lignophila Dill, Ramírez & González (1984)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal, measuring (2.7–5)×(4–10) µm, and may be single, in pairs or short chains or clusters.

A few elongate cells may occur (up to 30 µm). Cells are encapsulated. After one month, a sediment is present.

Growth on 5% malt extract agar: After one month at 25°C, the streak colony is white, the texture is butyrous, the cross section is flat, surface is dull and the margin is entire. At 19°C, the streak colony is cream-colored, mucoid and glistening.

Dalmau plate culture on corn meal agar: After one week at 19°C, pseudomycelium is absent (Fig. 414).

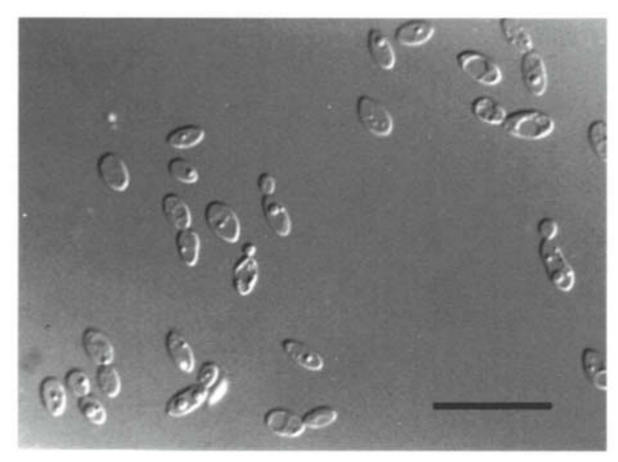


Fig. 414. *R. lignophila*, CBS 7109. Cells grown on corn meal agar after 5 days at 25°C. Bar = 15 µm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	s
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	s	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	s
D-Xylose	s	Succinate	w
L-Arabinose	–	Citrate	s
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	s	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	s	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G + C: Not determined.
Cell hydrolyzates: Xylose, absent; fucose and rhamnose, present (Roeijmans et al. 1989).

Origin of the strains studied: From trunks of the woody plant, *Drimys winteri,* that were in advanced stages of white rot decay (palo podrido) on the isle of Chiloé in South Chile. The habitat has a constant low temperature (annual mean temperature of 10–12°C) and high humidity (annual mean rainfall 2000–3000 mm) (Dill et al. 1984).

Type strain: CBS 7109 (Dill et al. 1984, strain S7/20).

105.25. *Rhodotorula marina* Phaff, Mrak & Williams (1952)

Growth in 5% malt extract: After 3 days at 25°C, the cells are single or in pairs and ovoidal to globose, (2–4)×(4.0–10.1) µm. A thin ring begins to form.

Growth on 5% malt extract agar: After one month at 25°C, the streak colony is orange-pink, glistening, smooth. The texture is pasty to soft. The cross-section is convex, the border entire.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	w	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	ws
Trehalose	s	Galactitol	+
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	w	α-Methyl-D-glucoside	ws
Melezitose	+	Salicin	w
Inulin	–	D-Gluconate	ws
Soluble starch	w	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	w	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Urease	+
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	PABA-free	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 25°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	–		
Starch formation	–		

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G + C: 53.9 (T_m: Sugiyama et al. 1985).
Cell hydrolyzates: Mannose dominant, hexitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Surface washings of shrimp in Aransas Bay near Rockport, Texas, January 1950 (Phaff et al. 1952).

Type strain: CBS 2365 (strain 48-23P of Phaff et al. 1952).

Comments: Based on standard taxonomic tests, this species was considered to be a synonym of *R. minuta* (Fell et al. 1984b, Barnett et al. 1990). However, nucleotide sequence analysis of a region of the LSU rRNA demonstrated that the two species are distinct, differing by 53 base positions (Fell et al. 1992). Therefore, we reestablished *R. marina* as a separate species. *R. marina* differs from *R. minuta* on the following: *R. marina* assimilates L-rhamnose, galactitol and citrate and does not utilize either *N*-acetyl-D-glucosamine, 2-keto-D-gluconate or 5-keto-D-gluconate as sole sources of carbon. Based on standard taxonomic tests, *R. marina* is difficult to distinguish from *R. mucilaginosa*; results of testing for growth at 30°C are positive for *R. mucilaginosa,* negative for *R. marina.* Based on nucleotide sequence analysis, *R. marina* and *R. mucilaginosa* are distinct species (Fell et al. 1992). Mol% G+C differs for the two species (*R. marina*, 54; *R. mucilaginosa*, 59–63). We listed growth on maltose and lactose as variable, which is based on the report of Phaff and Ahearn (1970) that the assimilation of those carbon compounds occurs after adaptation and is unstable; also, they listed D-arabinose as positive but latent.

105.26. *Rhodotorula minuta* (Saito) F.C. Harrison (1928)

Synonyms:
Torula minuta Saito (1922)
Torulopsis minuta (Saito) Ciferri & Redaelli (1925)
Mycotorula muris Ciferri & Redaelli (1925)
Proteomyces muris (Ciferri & Redaelli) Dodge (1935)
Rhodotorula texensis Phaff, Mrak & Williams var. *minuta* (Saito) Hasegawa, Banno & Yamauchi (1960)
Rhodotorula pallida Lodder (1934)
Rhodotorula texensis Phaff, Mrak & Williams (1952)
Rhodotorula minuta (Saito) F.C. Harrison var. *texensis* (Phaff, Mrak & Williams) Phaff & Ahearn (1970)
Rhodotorula tokyoensis Kobayashi (1953)
Rhodotorula laryngis Reiersöl (1955)
Rhodotorula slooffiae Novák & Vörös-Felkai (1962)
Rhodotorula zsoltii Galgóczy & Novák (1965)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal, globose or elongate, (2.3–4.5)×(3.5–6.5) µm, single and in pairs. There is a thin, pink ring and a little sediment. After one month, a moderate orange to red ring and a light sediment are present.

Growth on 5% malt agar: The cell morphology is similar to that in malt extract. After one month at 25°C, the streak culture is pink, smooth, glossy, soft but not mucoid, the cross section is flat to low convex and the margin entire.

Dalmau plate culture on corn meal agar: Pseudomycelium is absent.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	s	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
5-Keto-D-gluconate	+	Gelatin liquefaction	–
Saccharate	–	PABA-free	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	v
10% NaCl/5% glucose	–	Growth at 37°C	v
Starch formation	–		

Co-Q: 10 (Hamamoto et al. 1987).

Mol% G+C: 51.0–54.0 (BD: Storck et al. 1969; T_m: Nakase and Komagata 1971c); 48.2–50.1 (T_m: Hamamoto et al. 1987).

Cell hydrolyzates: Mannose dominant, hexitol (probably mannitol), pentitol (probably arabinitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: 151 strains from Japan, Europe, North America, New Zealand, Antarctica. Sources include: air (1), clinical (23), terrestrial plants and animals (8), marine water, plants and animals (115), pickles (1), fresh water (1), unknown sources (3).

Type strain: CBS 319, isolated by Saito from the atmosphere in Tokyo.

Comments: This species is prevalent and has a widespread occurrence on a variety of substrates. However, the physiological variability as expressed in the standard tests and the differences in mol% G+C values, suggest that strains identified as *R. minuta* may represent a diversity of species. This observation should be tested by nucleotide sequence analysis.

105.27. *Rhodotorula mucilaginosa* (Jörgensen) F.C. Harrison (1928)

Synonyms:
Saccharomyces ruber Demme (1889)
Cryptococcus ruber (Demme) Vuillemin (1901)
Torulopsis rubra (Demme) de Almeida (1933)
Rhodotorula rubra (Demme) Lodder (1934)
Rhodotorula rubra (Demme) Lodder var. *longa* Lodder (1934)
Rhodotorula rubra (Demme) Lodder var. *curvata* Lodder (1934)
Torula mucilaginosa Jörgensen (1909)
Torulopsis mucilaginosa (Jörgensen) Ciferri & Redaelli (1925)
Saccharomyces glutinis Carbone (Dodge 1935)

Torula sanguinea Schimon (1911)
Torulopsis sanguinea (Schimon) Ciferri & Redaelli (1925)
Rhodotorula sanguinea (Schimon) F.C. Harrison (1928)
Rhodotorula mucilaginosa (Jörgensen) F.C. Harrison var. *sanguinea* (Schimon) Lodder (1934)
Rhodotorula rubella F.C. Harrison (1928)
Cryptococcus ludwigi Anderson (1918)
Rhodotorula ludwigii (Anderson) Krasil'nikov (1954b)
Torula corallina Saito (1922)
Torulopsis corallina (Saito) Ciferri & Redaelli (1925)
Rhodotorula corallina (Saito) F.C. Harrison (1927)
Cryptococcus mena Fontoynont & Boucher (1923)
Torulopsis mena (Fontoynont & Boucher) Dodge (1935)
Blastodendrion carbonei Ciferri & Redaelli (1925)
Rhodotorula mucilaginosa (Jörgensen) F.C. Harrison var. *carbonei* Lodder (1934)
Torulopsis mucilaginosa (Jörgensen) Ciferri & Redaelli var. *carbonei* (Ciferri & Redaelli) Dodge (1935)
Eutorulopsis dubia Ciferri & Redaelli (1925)
Mycotorula pulmonalis Ciferri & Redaelli (1925)
Geotrichum pulmonale (Ciferri & Redaelli) Dodge (1935)
Torulopsis sanniei Ciferri & Redaelli (1925)
Cryptococcus sanniei (Ciferri & Redaelli) Nannizzi (1934)
Rhodotorula sanniei (Ciferri & Redaelli) Lodder (1934)
Torulopsis biourgei Ciferri & Redaelli (1925)
Rhodotorula biourgei (Ciferri & Redaelli) Krasil'nikov (1954b)
Blastodendrion simplex Ciferri & Redaelli (1925)
Cryptococcus simplex (Ciferri & Redaelli) Nannizzi (1934)
Cryptococcus rubrorugosus Castellani (1927)
Cryptococcus pararoseus Castellani (1927)
Rhodotorula mucilaginosa (Jörgensen) F.C. Harrison var. *pararosea* (Castellani) Lodder (1934)
Torulopsis mucilaginosa (Jörgensen) Ciferri & Redaelli var. *pararosea* (Lodder) Dodge (1935)
Torula aclotiana F.C. Harrison (1928)
Rhodotorula aclotiana F.C. Harrison (1928)
Cryptococcus corallinus Sartory, Sartory, Hufschmitt & J. Meyer (1930)
Sporobolomyces alborubescens Derx (1930)
Sporobolomyces photographus Ciferri & Redaelli var. *alborubescens* (Derx) Verona & Ciferri (1938)
Torulopsis nitritophila Ciferri & Ashford (Ashford and Ciferri 1930)
Cryptococcus radiatus A. & R. Sartory & J. Meyer (1931)
Torula decolans Okunuki (1931)
Torulopsis mannitica Castelli (1932)
Torulopsis aurantia Zach (Wolfram and Zach 1934b)
Rhodotorula mucilaginosa (Jörgensen) F.C. Harrison var. *plicata* Lodder (1934)
Torulopsis mucilaginosa (Jörgensen) Ciferri & Redaelli var. *plicata* (Lodder) Dodge (1935)
Rhodotorula matritense Ramírez, González & Gutierrez (1981)
Rhodotorula mucilaginosa (Jörgensen) F.C. Harrison var. *kentuckyi* Giovannozzi (1948)
Rhodotorula pilimanae Hedrick & Burke (1951)
Rhodotorula vuilleminii Saëz (1967)
Rhodotorula ulzamae Moriyon & Ramírez (1974)
Rhodotorula rubra (Demme) Lodder var. *marina* Kawano, Kojima, Ohosawa & Morinaga (1976)
Rhodotorula grinbergsii Ramírez & González (1984b)
Mycotorula cisnerosi nom. nud. (cf. Fell et al. 1984b)
Mycotorula germanica nom. nud. (cf. Fell et al. 1984b)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to spherical, measuring (2–8)×2–12) µm; reproduction is by multilateral budding and cells may occur singly, in pairs, in short chains or small clusters. There may be a light strawberry-pink ring and a light to moderate sediment. After one month, there may be islets and a light to heavy ring that is pink to salmon-colored with a moderate to heavy sediment.

Growth on 5% malt extract agar: After one month at 25°C, the streak colony color varies from saffron-orange, pink-salmon to deep coral. The cross section is flat, the surface varies from smooth to rugoid, and dull to glistening. The margin is entire.

Dalmau plate culture on corn meal agar: After one month at 19°C, pseudomycelium is absent or rudimentary, consisting of chains of elongate cells.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	v	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	v
Lactose	–	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	–
L-Rhamnose	v	Nitrate	–
D-Glucosamine	v	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Starch formation	–
5-Keto-D-gluconate	–	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	v	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	+
10% NaCl/5% glucose	v		

Co-Q: 10 (Hamamoto et al. 1987).

Mol% G+C: 59.0–59.7 (T_m: Hamamoto et al. 1987); 60–63.5 (T_m: Nakase and Komagata 1968a, 1971c, Marmur and Doty 1962).

Cell hydrolyzates: Fucose, mannose dominant, hexitol (probably mannitol), pentitol (probably arabinitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Powdered red pepper, *Capsicum frutescens,* Spain (1); larvae of fruit fly (*Drosophila pilimanae*), Hawaii (1); rotten *Eucryphia cordifolia,* Chile (1); smut infected leaves in southern France (1). Of the 32 strains studied for the original description of *R. mucilaginosa,* 10 were isolated from humans, pasteurized beer, air in Tokyo and Italy.

Type strain: CBS 316. This strain was supplied to CBS in 1934 by the National Collection of Type Cultures in London, which obtained it from Bierberg (Germany) in 1912 as the type strain of *R. mucilaginosa* (Lodder 1970).

Comments: *Rhodotorula mucilaginosa* has a world-wide distribution in terrestrial, aquatic and marine habitats,

from a wide variety of substrates. The species is frequently isolated from human sources, and *R. mucilaginosa* is possibly the only species of *Rhodotorula* that causes human infections (Kwon-Chung and Bennett 1992). Sexual cycles in *R. mucilaginosa* have not been reported. We tested a large number of strains from marine environments for mating responses and we did not observe any positive results.

In the last two editions of "The Yeasts", *R. mucilaginosa* was included as a synonym of *R. rubra*. According to Barnett et al. (1990), the epithet *R. mucilaginosa* has priority and the reason is as follows: Demme (1889) described *Saccharomyces ruber* based on CBS 17. Vuillemin (1901) transferred the species to *Cryptococcus*, then Lodder (1934) created the combination *Rhodotorula rubra*. In the meantime, Saito (1923) described *Torulopsis rubra* based on CBS 2366, a species that Harrison (1928) transferred to *Rhodotorula* as *R. rubra* (Saito) Harrison. CBS 2366, however, was found to be a strain of *Rhodotorula glutinis* (Barnett et al. 1990) and it is taxonomically distinct from CBS 17 (*Rhodotorula rubra* Lodder). Because *R. rubra* (Saito) Harrison was the first usage of that species epithet in the genus, the name is no longer available. Hence, *R. rubra* Lodder cannot be used. Because CBS 17 and the type strain of *R. mucilaginosa* (CBS 316) are taxonomically identical (Barnett et al. 1990, Fell et al. 1992), the nomenclature, *R. mucilaginosa*, is correct.

R. mucilaginosa has a long list of synonyms, which is due to the variability among strains in the utilization of the majority of the test carbon compounds. Some of this strain variability could signify separate species, a possibility that should be examined by nucleotide sequence analysis. Our examination (Fell et al. 1992) of a region of the LSU rRNA from the type strains of *Sporobolomyces alborubescens*, *R. grinbergsii*, *R. rubra*, *R. pilimanae*, *R. matritensis* and *R. mucilaginosa* demonstrated identical sequence alignments, which indicates synonymous species among these taxa. Our inclusion of *S. alborubescens* is at variance with Boekhout's (1991a) retention of that species in *Sporobolomyces*. The only report of ballistoconidia in *S. alborubescens* is found in the original description. Our attempts to induce ballistoconidia have not been successful with that strain or other strains of *R. mucilaginosa*.

Barnett et al. (1990) reported variable reactions in nitrate assimilation. The positive reactions were attributed to a report by Hamamoto et al. (1987) who found high (66–82%) relative binding of DNA between three strains (YK 114, 115, 116) of *R. glutinis* and the type strain *R. rubra*, but not between the type strains of the two species. Our inspection of their data suggested that the three YK strains belong to *R. rubra* rather than *R. glutinis*. Hamamoto et al. did not present the nitrate assimilation data for their test strains, however, two of the strains (YK 115 and 116) demonstrated low (33–34)% relative binding with the type strain of *R. glutinis*. The third strain YK 114, which was tested against YK 115, resulted in 99% binding. Because we have not observed nitrate-assimilating strains of *R. mucilaginosa* and because we find that strains of the two species are significantly different in nucleotide sequence alignments (Fell et al. 1992), nitrate-assimilating strains are not included in the species description.

105.28. *Rhodotorula muscorum* (di Menna) von Arx & Weijman (1979)

Synonyms:

Candida muscorum di Menna (1958a)

Azymocandida muscorum (di Menna) Novák & Zsolt (1961)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal to irregularly elongate, (2–3)×(5–10.7) µm. After one month, a ring and heavy sediment are present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is yellowish cream-colored, semi-glistening, mucoid, soft, and smooth; warty outgrowths may be present.

Dalmau plate culture on corn meal agar: After 7 days at 19°C, pseudomycelium, consisting of ramified chains of cells bearing few blastoconidia, is present.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	s
Trehalose	+	Galactitol	s
Lactose	s	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	s	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+	Vitamin-free	s

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	+	Gelatin liquefaction	–
D-Glucuronate	+	Thiamine-free	–
50% (w/w) Glucose–yeast extract agar	–	Growth at 25°C	+
		Growth at 30°C	–
10% NaCl/5% glucose	+		

Co-Q: 10 (Sugiyama et al. 1985).

Mol% G + C: Not determined.

Cell hydrolyzates: Fucose and mannose are dominant; hexitol (probably mannitol) and pentitol (probably arabinitol) are present as well (Weijman and Rodrigues de Miranda 1988). According to Sugiyama et al. (1985), xylose is present.

Origin of the strains studied: Rotting layer of sphagnum overlying a peat bog at Paraparaumu in the Wellington district, New Zealand and forest soils from Chatham Island, 700 miles east of New Zealand (di Menna 1958a) (1); barley leaf (1).

Type strain: CBS 6921. Meyer et al. (1984) reported that the type strain, CBS 2912 (di Menna strain PS2) no longer agreed with the description and was replaced with CBS 6921. Both cultures were derived from the di Menna strains.

Comments: Based on standard carbon and nitrogen utilization tests, this species resembles *R. fragaria*. The two species are distinct and can be separated taxonomically by the following: *R. muscorum* assimilates saccharate and slowly assimilates galactitol; *R. fragaria* does not assimilate either compound. *R. muscorum* does not assimilate L-arabinose, *R. fragaria* assimilates it slowly. Nucleotide sequence analysis in our laboratory (unpublished) indicates that *R. fragaria, R. muscorum* and *Leucosporidium scottii* are closely related, yet distinct species.

105.29. *Rhodotorula nothofagi* (Ramírez & González) Roeijmans, van Eijk & Yarrow (1989)

Synonym:
Apiotrichum nothofagi Ramírez & González (1984d)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal to elongate, (2–6)×(4.7–8.7) μm, single or in pairs. After one month, islets and a heavy sediment are present.

Growth on 5% malt extract agar: After one month at 25°C, the streak colony is light yellowish-pink, smooth, slightly glistening; the texture is butyrous, the cross section is flat, and the margin is entire.

Dalmau plate culture on corn meal agar: After one week at 19°C, pseudomycelium is absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	–	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	w
L-Arabinose	+	Citrate	w
D-Arabinose	s	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	+	Growth at 37°C	–
10% NaCl/5% glucose	s		

Co-Q: Not determined.
Mol% G + C: Not determined.
Cell hydrolyzates: Xylose absent, fucose and rhamnose positive (Roeijmans et al. 1989).

Origin of the strain studied: Ramírez and González (1984d) isolated a single strain of this species in 1980 from decayed wood of *Nothofagus obligua* (Mirb.) Blume in the evergreen rainy Valdivian forest of southern Chile, near the locality of Futrono.

Type strain: CBS 8166 (Ramírez and González number IFJM 6018).

Comments: Ramírez and González (1984d) reported that L-rhamnose was assimilated and that D-xylose, L-arabinose, D-arabinose, and D-ribose were not assimilated; we were unable to confirm these results.

105.30. *Rhodotorula philyla* (van der Walt, van der Klift & D.B. Scott) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonyms:
Torulopsis philyla van der Walt, van der Klift & D.B. Scott (van der Walt et al. 1971b)
Candida philyla (van der Walt, van der Klift & D.B. Scott) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)
Vanrija philyla (van der Walt, van der Klift & D.B. Scott) R.T. Moore (1980)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ellipsoidal to ovoidal, (2–4.7)×(3.3–6) μm, and occur singly, in pairs, or in chains. A thin ring and sediment are present. After one month at 20°C, a ring and sediment are present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is cream-colored, glistening, smooth, mucoid, and the margin is entire.

Dalmau plate culture on corn meal agar: After one week at 19°C, no pseudomycelium is formed.

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	–
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	s
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 30°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 37°C	–
10% NaCl/5% glucose	–		

Co-Q: 10 (Sugiyama et al. 1985).
Mol% G+C: 62.9, type strain (T_m: Meyer et al. 1984).
Cell hydrolyzates: Fucose, rhamnose, mannose dominant, hexitol (probably mannitol) and pentitol (probably arabinitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Tunnels of the beetle *Xyleborus ferrugineus* F. infecting an indigenous tree, *Harpephyllum caffrum* Bernh. ex Krass, near Mtunzini, Natal (van der Walt et al. 1971b).

Type strain: CBS 6272.

105.31. *Rhodotorula phylloplana* (Shivas & Rodrigues de Miranda) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonym:
Cryptococcus phylloplanus Shivas & Rodrigues de Miranda (1983a)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal to cylindrical, (2.3–3.3)×(4.7–9.4) μm, and occur singly or in pairs. After one month, a very light ring and sediment have formed.

Growth on 5% malt extract agar: After one month at 19°C, the streak colony is buff-colored, smooth, semi-glistening, butyrous, and the margin is entire.

Dalmau plate culture on corn meal agar: After one week at 19°C, pseudo- and true mycelium have not formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	–	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	s
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G+C: Not determined.
Cell hydrolyzates: Mannose low, malic acid (Weijman and Rodrigues de Miranda 1988).

Origin of the strain studied: Surface of leaves of the honeysuckle, *Banksia collina* R. Br., growing at Armidale, New South Wales, Australia, collected in 1981 (Shivas and Rodrigues de Miranda 1983a).

Type strain: CBS 8073.

Comments: *R. phylloplana* and *R. hinnulea* are the only species in the genus that demonstrate growth on inositol. The two species can be separated by their differences in utilizing cellobiose and trehalose. Shivas and Rodrigues de Miranda (1983a) reported that cellobiose, trehalose and soluble starch were assimilated weakly, and xylose, salicin and DL-lactic acid were assimilated strongly; we were unable to confirm these results. Nucleotide sequence analysis in our laboratory (unpublished) indicates that *R. phylloplana* is related to the Ustilaginales. This relationship is also suggested by the low level of mannose in the cell hydrolyzates, which is characteristic of the smut-like yeasts.

105.32. *Rhodotorula pilati* (F.H. Jacob, Faure-Raynaud & Berton) J.A. Barnett, Payne & Yarrow (1983)

Synonym:
Torulopsis pilati F.H. Jacob, Faure-Raynaud & Berton (1979)

Growth in 5% malt extract: After 3 days at 25°C, the cells are ovoidal, single or in pairs, measuring (4.0–6.0)×(5.0–12.7) μm. After one month a sediment and a ring have formed.

Growth on 5% malt extract agar: After one month at 25°C, the colony is cream-colored, the cross section

is raised, the surface is glistening and smooth, and the margin is entire.

Dalmau plate culture on corn meal agar: After one month at 25°C, neither true nor pseudomycelium is present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G + C: Not determined.
Cell hydrolyzates: Fucose, rhamnose, mannose dominant, hexitol (probably arabinitol) and pentitol (probably mannitol) (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Leaf litter of fir tree *Abies alba* located at an altitude of 1100 m at Massif du Pilat, Loire, France (Jacob et al. 1979); decaying tree trunk, Portugal.

Type strain: CBS 7039 (strain LYJ 382 of Jacob et al. 1979).

Comments: The original description stated that nitrate was not assimilated, that growth occurred in the absence of vitamins and that α-methyl-D-glucoside was utilized as a sole carbon source, characteristics that we were unable to confirm.

105.33. *Rhodotorula pustula* (Buhagiar) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonyms:
Torulopsis pustula Buhagiar (1975)
Candida pustula (Buhagiar) S.A. Meyer & Yarrow (Yarrow and Meyer 1978)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ovoidal, long-ovoidal to cylindrical, and pear-shaped, (3.4–5.6) × (8.4–10.6) μm. After one month a ring and sediment are present.

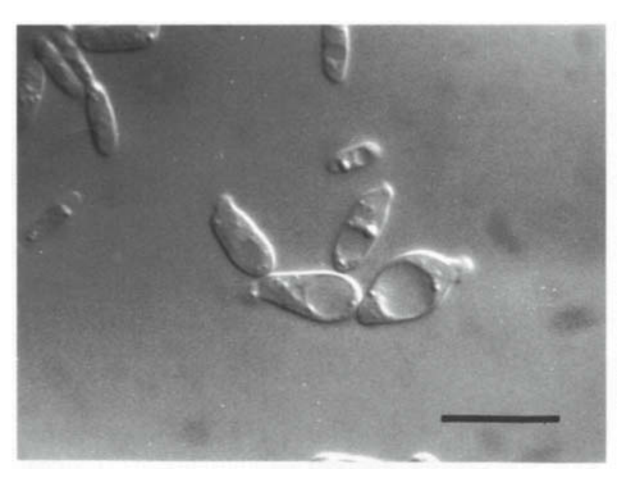


Fig. 415. *R. pustula*, CBS 6527. Cells grown on corn meal agar at 25°C after 5 days. Bar = 10 μm.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak is white to tan-white, mucoid, glossy, smooth, and convex with an entire margin.

Dalmau plate culture on corn meal agar: Branched chains of long, ovoidal cells are formed with short chains of blastoconidia (Fig. 415).

Fermentation: absent.

Assimilation of carbon compounds:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	–	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	s	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	n	Starch formation	–
5-Keto-D-gluconate	n	Urease	+
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	+	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.
Mol% G + C: 51.0, type strain (T_m: Meyer et al. 1984).
Cell hydrolyzates: Fucose, rhamnose, mannose dominant, hexitol (probably arabinitol and pentitol (probably mannitol) present (Weijman and Rodrigues de Miranda 1988).

Origin of the strains studied: Black currants (*Ribes nigrum* cultivar Baldwin) collected in 1971 and 1972 in England.

Type strain: CBS 6527.

Comments: The original description noted that hexadecane utilization was delayed, a characteristic that we were unable to confirm.

105.34. *Rhodotorula sonckii* (Hopsu-Havu, Tunnela & Yarrow) Rodrigues de Miranda & Weijman (Weijman et al. 1988)

Synonym:
Candida sonckii Hopsu-Havu, Tunnela & Yarrow (1978)

Growth in glucose–yeast extract–peptone water: After 3 days at 25°C, the cells are ellipsoidal to cylindrical, (2.5–4.0)×(7.0–12.0) µm, single or in pairs. A sediment is formed. After one month at room temperature, a sediment is present.

Growth on glucose–yeast extract–peptone agar: After one month at 25°C, the streak culture is cream-colored, smooth, glistening and flat. The margins are entire. Growth is slow.

Dalmau plate culture on corn meal agar: After one week, pseudomycelium is not formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	s	Ethanol	–
Sucrose	–	Glycerol	s
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	–	Urease	v
Saccharate	–	Gelatin liquefaction	–
D-Glucuronate	–	Growth at 25°C	+
50% (w/w) Glucose–yeast extract agar	–	Growth at 30°C	–
10% NaCl/5% glucose	–		

Co-Q: Not determined.

Mol% G+C: 44.1 (T_m: Hopsu-Havu et al. 1978).

Cell hydrolyzates: Fucose, mannose dominant (Weijman & Rodrigues de Miranda 1988).

Origin of the strains studied: Wall of a bathing pool during a routine check of hospital hygiene in the pediatric department of the University Hospital of Turku, Finland in Oct. 1973 (CBS 6713, Hopsu-Havu et al. 1978); from the arm of a patient in Finland (CBS 8115), strain submitted to CBS by Hopsu-Havu.

Type strain: CBS 6713.

Comments: The original description noted that ribitol could not support growth as the sole source of carbon, a characteristic that we were unable to confirm.

Comments on the genus

Species in the genus *Rhodotorula* date to Fresenius (1850) with the description of *Cryptococcus glutinis.* The genus was formally described by Harrison (1928) to include red pigmented asporogenous yeasts. Other pigmented yeasts were placed in *Chromotorula.* The latter genus was rejected by Lodder (1934) who placed all of the carotenoid species in *Rhodotorula.* In the first edition of *The Yeasts, A Taxonomic Study* (Lodder and Kreger-van Rij 1952), *Rhodotorula* was distinguished as occasionally having a primitive pseudomycelium, producing carotenoid pigments and lacking the ability to ferment. Seven species were accepted. There was little change in the generic descriptions through the 2nd edition of "*The Yeasts*" with the exception that *Rhodotorula* species were described as not having the capacity to assimilate inositol nor synthesize starch-like compounds. Nine species were recorded in the genus. The major informative step, by this time, was the confirmation of the basidiomycetous nature of this genus, particularly the observations of teleomorphic stages described in the genus *Rhodosporidium.* The next major change was a result of an altered definition of *Candida.* Ascomycetous species were retained in *Candida* and the basidiomycetous species were separated based on the composition of cell hydrolyzates (Weijman and Rodrigues de Miranda 1983). Species with xylose in the cell hydrolyzates were placed in *Cryptococcus* and species which lacked xylose were relegated to *Rhodotorula.*

The genus, as currently defined, includes 34 species that comprise a polyphyletic group of organisms. The separation of these species is based on 2 morphological characters, 19 physiological tests and one growth temperature test. To eliminate some of these tests, we present Tables 86–91, which employ a reduced number of characteristics. These tables include the teleomorphic species that could be identified with these tests.

Our suggestion for an orderly identification procedure includes a preliminary screening with the following tests: assimilation of inositol, D-glucuronate and nitrate and the synthesis of starch. After comparing the results with Table 85, the next step is to examine the appropriate Table and use those tests. It is also valuable, in the initial stages of identification, to examine colony color, cell morphology and potential formation of mycelium and teliospores. Through the use of these identification shortcuts, it should not be necessary, for routine identifications, to employ all of the tests listed in Table 86. Mating tests

should be undertaken when multiple strains of a species are available, because many of the *Rhodotorula* species are anamorphic strains of described and undescribed teleomorphic species. These mating tests consist of mixing multiple strains together on a complex nutrient medium such as corn meal agar. If mating occurs, the number of strains can be reduced in subsequent crosses to find the mating pairs.

Our laboratory is currently studying the molecular systematics of this genus. Some of the unpublished results, which were obtained after the completion of this manuscript, are included in the species comments. The results indicate diverse phylogenetic origins of species in the genus and that closely related anamorphic species include members of the genera *Bensingtonia, Sporobolomyces* and *Sterigmatomyces.* Some species, particularly *R. acheniorum, R. bacarum, R. hinnulea* and *R. phylloplana,* may be related to the smut-like species in the Ustilaginales. In the near future, we plan to present an integrated view of the genus based on molecular and phenotypic data, which we hope will eliminate some of the taxonomic problems in *Rhodotorula.*

Species not examined: The following species were not included because they came belatedly to the attention of the authors:

Rhodotorula cresolica Middelhoven and Spaaij (1997)
Rhodotorula vanillica J.P. Sampaio (1994)

106. *Sporobolomyces* Kluyver & van Niel

T. Boekhout and T. Nakase

Diagnosis of the genus

Cells are ellipsoidal, subglobose, fusiform or cylindroidal. Budding is mostly polar, rarely lateral or multilateral, with buds sessile or on short denticles, enteroblastic, and with percurrent or sympodial proliferation. Ballistoconidia are bilaterally symmetrical, ellipsoidal, falcate, allantoid, lacrymoid or amygdaliform. Colonies are salmon-pink, orange-red, red, cream, or yellow-brown. Hyphae and pseudohyphae may be present.

Sugars are not fermented. Diazonium blue B and urease reactions are positive. Xylose is absent from whole-cell hydrolyzates. The major ubiquinone is Q-10 or Q-10(H_2).

Type species

Sporobolomyces salmonicolor (Fischer & Brebeck) Kluyver & van Niel

Species accepted

1. *Sporobolomyces alborubescens* Derx (1930)
2. *Sporobolomyces elongatus* Shivas & Rodrigues de Miranda (1983)
3. *Sporobolomyces falcatus* Nakase, M. Itoh & M. Suzuki (1987)
4. *Sporobolomyces foliicola* Shivas & Rodrigues de Miranda (1983)
5. *Sporobolomyces gracilis* Derx (1930)
6. *Sporobolomyces griseoflavus* Nakase & M. Suzuki (1987)
7. *Sporobolomyces inositophilus* Nakase & M. Suzuki (1987)
8. *Sporobolomyces kluyveri-nielii* van der Walt (1986)
9. *Sporobolomyces lactophilus* Nakase, M. Itoh, M. Suzuki & Bandoni (1990)
10. *Sporobolomyces oryzicola* Nakase & M. Suzuki (1986)
11. *Sporobolomyces phyllomatis* van der Walt & Y. Yamada (1988)
12. *Sporobolomyces roseus* Kluyver & van Niel (1924)
13. *Sporobolomyces ruber* (Nakase, Okada & Sugiyama) Boekhout (1991)
14. *Sporobolomyces salicinus* (Johri & Bandoni) Nakase & M. Itoh (1988)
15. *Sporobolomyces salmonicolor* (Fischer & Brebeck) Kluyver & van Niel (1924): see *Sporidiobolus johnsonii,* p. 694
16. *Sporobolomyces sasicola* Nakase & M. Suzuki (1987)
17. *Sporobolomyces shibatanus* (Okunuki) Verona & Ciferri (1938): see *Sporidiobolus pararoseus,* p. 694
18. *Sporobolomyces singularis* Phaff & do Carmo-Sousa (1962)
19. *Sporobolomyces subbrunneus* Nakase & M. Suzuki (1985)
20. *Sporobolomyces tsugae* (Phaff & do Carmo-Sousa) Nakase & M. Itoh (1988)
21. *Sporobolomyces xanthus* (Nakase, Okada & Sugiyama) Boekhout (1991)

Key to species

See Table 92.

1.	a	Self-sporulating formation of teliospores present	see *Sporidiobolus*:	p. 693
	b	Self-sporulating formation of teliospores absent → 2		
2(1).	a	Nitrate assimilated → 3		
	b	Nitrate not assimilated → 17		
3(2).	a	Sucrose assimilated → 4		
	b	Sucrose not assimilated → 15		
4(3).	a	D-Ribose assimilated → 5		
	b	D-Ribose not assimilated → 8		
5(4).	a	Ethanol assimilated → 6		
	b	Ethanol not assimilated → 7		
6(5).	a	Strain mates with mating strain of *Sporidiobolus salmonicolor*	*S. salmonicolor*:	p. 839
	b	Strain does not mate with mating strain of *Sporidiobolus salmonicolor*	*S. roseus*:	p. 836
7(5).	a	Maltose assimilated	*S. roseus*:	p. 836
	b	Maltose not assimilated	*S. foliicola*:	p. 831
8(4).	a	Maltose assimilated → 9		
	b	Maltose not assimitated → 14		
9(8).	a	Raffinose assimilated → 10		
	b	Raffinose not assimilated → 11		

Table 92
Key characters of species in the genera *Sporidiobolus* and *Sporobolomyces*

Species	Teliospore formation[a]		Assimilation[b]											
	Self	Hetero	Gal	Suc	Mal	Cel	Lac	Raf	Ara	Rib	Eth	α-M	Sal	Nit
Sporidiobolus johnsonii	+	−	v	+	+	+	−	−	v	+	+	+	x[c]	+
S. pararoseus	+	+	+	+	+	+	−	+	−	v	+	+	+	−
S. ruineniae	+	−	+	+	v	+	−	+	+	+	+	−	+	+
S. salmonicolor	−	+	v	+	v	v	−	v	v	+	+	v	v	+
Sporobolomyces alborubescens	−	−	+	+	+	+	−	+	+	v	+	−	+	−
S. elongatus	−	−	−	+	+	+	−	−	+	−	−	−	+	−
S. falcatus	−	−	w	−	−	+	−	−	−	−	+	−	+	+
S. foliicola	−	−	+	+	−	w	−	−	+	+	−	−	−	+
S. gracilis	−	−	−	−	−	+	−	−	−	+	−	−	−	−
S. griseoflavus	−	−	+	+	+	+	−	−	−	−	+	−	+	+
S. inositophilus	−	−	−	+	+	−	−	−	−	−	+	−	−	+
S. kluyveri-nielii	−	−	+	−	−	−	−	−	+	−	+	−	−	+
S. lactophilus	−	−	−	+	+	+	+	−	−	−	v	v	−	+
S. oryzicola	−	−	+	+	+	+	−	+	+	+	−	+	w	−
S. phyllomatis	−	−	+	+	+	+	−	−	+	−	+	−	−	−
S. roseus	−	−	v	v	+	v	−	+	v	v	v	v	v	+
S. ruber	−	−	−	+	−	−	−	+	−	−	−	+	−	−
S. salicinus	−	−	+	+	−	+	−	+	+	−	−	−	w	+
S. sasicola	−	−	−	+	+	+	+	+	+	−	−	−	w	−
S. singularis	−	−	−	−	−	+	+	−	v	−	+	−	+	−
S. subbrunneus	−	−	−	+	−	−	−	+	−	−	−	−	−	+
S. tsugae	−	−	−	+	+	+	+	−	−	−	+	v	+	+
S. xanthus	−	−	w	+	+	+	w	+	+	−	−	+	−	+

[a] Self, teliospore formation by self-sporulation; hetero, teliospore formation by mating.
[b] Abbreviations: Gal, galactose; Suc, sucrose; Mal, maltose; Cel, cellobiose; Lac, lactose; Raf, raffinose; Ara, L-arabinose; Rib, D-ribose; Eth, ethanol; α-M, α-methyl-D-glucoside; Sal, salicin; Nit, nitrate.
[c] Positive or weak.

10(9). a Lactose weakly assimilated .... *S. xanthus*: p. 841
b Lactose not assimilated .... *S. roseus*: p. 836
11(9). a Cellobiose assimilated → 12
b Cellobiose not assimilated .... *S. inositophilus*: p. 833
12(11). a Salicin assimilated → 13
b Salicin not assimilated .... *S. lactophilus*: p. 834
13(12). a Lactose assimilated .... *S. tsugae*: p. 841
b Lactose not assimilated .... *S. griseoflavus*: p. 832
14(8). a Cellobiose assimilated .... *S. salicinus*: p. 838
b Cellobiose not assimilated .... *S. subbrunneus*: p. 840
15(3). a Maltose assimilated .... *S. roseus*: p. 836
b Maltose not assimilated → 16
16(15). a Cellobiose assimilated .... *S. falcatus*: p. 831
b Cellobiose not assimilated .... *S. kluyveri-nielii*: p. 834
17(2). a Galactose assimilated → 18
b Galactose not assimilated → 21
18(17). a Raffinose assimilated → 19
b Raffinose not assimilated .... *S. phyllomatis*: p. 835
19(18). a α-Methyl-D-glucoside assimilated → 20
b α-Methyl-D-glucoside not assimilated .... *S. alborubescens*: p. 830
20(19). a L-Arabinose assimilated .... *S. oryzicola*: p. 835
b L-Arabinose not assimilated .... *Sporidiobolus pararoseus*: p. 694
21(17). a Cellobiose assimilated → 22
b Cellobiose not assimilated .... *S. ruber*: p. 837

22(21). a Maltose assimilated → 23
b Maltose not assimilated → 24
23(22). a Raffinose assimilated .... *S. sasicola*: p. 839
b Raffinose not assimilated .... *S. elongatus*: p. 830
24(22). a D-Ribose assimilated .... *S. gracilis*: p. 832
b D-Ribose not assimilated .... *S. singularis*: p. 839

Systematic discussion of the species

106.1. *Sporobolomyces alborubescens* Derx (1930)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal, (6.0–9.0)×(4.0–6.5) μm, and single. Budding is polar, and buds are sessile or on short denticles. Colonies are butyrous, smooth, glabrous, shiny, pinkish-red or red, and with the margin entire, straight or somewhat crenulate.

Growth on the surface of assimilation media (glucose): A ring, islets and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, part of the cells form short, irregular hyphal outgrowths. Aerobic growth is red (brick red), shiny, butyrous, smooth or weakly ridged or grooved, somewhat raised, and with the margin entire.

Formation of ballistoconidia: Ballistoconidia were not observed. According to Derx (1930) they measure 9.5–11.0×4 μm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	v	Salicin	+
Inulin	+	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Urease	+
50% (w/w) Glucose	v	Nitrite	–
Starch formation	–	Growth at 37°C	+

Co-Q: 10 (Nakase 1989).
Mol% G+C: 63.0, CBS 482 (BD: Storck et al. 1969).

Origin of the strains studied: CBS 482 (ATCC 24216, JCM 5352, NRRL Y-6683, UCD-FST 68-329), leaf infected with rusts, H.G. Derx, France; CBS 6239, dialysis equipment, M. Bruining, Netherlands.

Type strain: CBS 482.

Comments: The two strains of *S. alborubescens* studied have lost the capacity to form ballistoconidia. Therefore, the species may be confused with species of *Rhodotorula*. Physiologically closely related *Rhodotorula* species are *R. minuta* and *R. mucilaginosa*. *Rhodotorula minuta* differs by a lower mol% G+C of 50.2–54.5 (Storck et al. 1969, Nakase and Komagata 1971c). *Rhodotorula mucilaginosa* is very similar, but can be differentiated by its lack of growth on salicin.

106.2. *Sporobolomyces elongatus* Shivas & Rodrigues de Miranda (1983b)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal, cylindrical to subglobose, (6.0–16.0)×(3.0–9.0) μm, and single. Budding is polar or lateral, and buds are sessile or on short denticles. Colonies are butyrous, smooth, shiny, salmon-pink, and with the margin entire.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, hyphae and pseudohyphae are absent. Cells are ellipsoidal to cylindroidal. Aerobic growth is orange-pink to salmon, shiny, butyrous, smooth, with the center somewhat raised, and the margin entire.

Formation of ballistoconidia: Ballistoconidia were not observed. According to Shivas and Rodrigues de Miranda (1983b), they are kidney-shaped, (5.0–8.0)×(2.5–4.0) μm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	–
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10(H_2) (Nakase and Suzuki 1986d).
Mol% G+C: 56.5 (Nakase 1989).

Origin of the strain studied: CBS 8080 (ATCC 52908), leaf of *Callistemon viminalis,* J.F. Brown, Australia.

Type strain: CBS 8080.

Comments: *Sporobolomyces elongatus* differs from all other *Sporobolomyces* species by the presence of Co-Q 10(H_2) (Nakase and Suzuki 1986d). Ballistoconidium formation seems an easily lost feature of this species. *Rhodotorula minuta* is physiologically similar, but differs by its growth on ribitol and ethanol.

106.3. *Sporobolomyces falcatus* Nakase, M. Itoh & M. Suzuki (1987a)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal, (4.0–8.5)×(1.5–3.5) µm, and single. Budding is usually polar, but also lateral, and with buds sessile or on short denticles and with sympodial or percurrent proliferation. Cells may form branched hyphae. Colonies are butyrous, shiny or dull, cream or grayish-white, with the margin entire or somewhat eroded.

Growth on the surface of assimilation media (glucose): A ring and a sediment are formed.

Dalmau plate on morphology agar: After 7 days at 25°C, no hyphae or pseudohyphae occur. However, short hyphae are formed on malt extract agar. Aerobic growth is pale cream, shiny, butyrous, smooth and slightly striated near the margin, with the center somewhat raised, and the margin entire, straight or crenulate.

Formation of ballistoconidia: Ballistoconidia are formed on malt extract agar and corn meal agar. They are falcate or amygdaliform, (5.0–12.0)×(1.5–3.0) µm (Fig. 416).

Fermentation: absent.

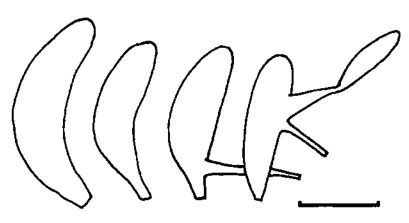


Fig. 416. *S. falcatus*, CBS 7368. Ballistoconidia on malt extract agar. Bar = 10 µm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 30°C	+
Starch formation	–	Growth at 37°C	–
Urease	+		

Co-Q: 10 (Nakase et al. 1987a).
Mol% G+C: 53.1, JCM 6838 (T_m: Nakase et al. 1987a).

Origin of the strain studied: CBS 7368 (ATCC 64693, JCM 6838), dead leaf of *Miscanthus sinensis*, T. Nakase, Japan.

Type strain: JCM 6838.

Comments: *Sporobolomyces falcatus* belongs to a group of species characterized by pale colonies and growth on 2-keto-D-gluconate ("*singularis* group" according to Nakase 1989). *Sporobolomyces falcatus* is characterized by growth on D-glucosamine, and lack of growth on sucrose, maltose and melezitose.

106.4. *Sporobolomyces foliicola* Shivas & Rodrigues de Miranda (1983b)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal to subglobose, (7.5–16.5)×(5.0–13.0) µm, and single. Budding is polar or multilateral, and with percurrent or sympodial proliferation. Colonies are butyrous, smooth, somewhat shiny, red (incarnate), and with the margin entire.

Growth on the surface of assimilation media (glucose): Islets and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, no hyphae or pseudohyphae are formed. Aerobic growth is red (incarnate), somewhat dull, butyrous, smooth, with the center somewhat raised, and the margin entire.

Formation of ballistoconidia: Ballistoconidia are formed on corn meal agar. They are ellipsoidal, (6.5–9.5)×(4.0–) µm (Fig. 417).

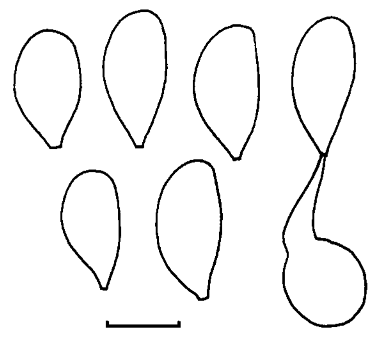


Fig. 417. *S. foliicola,* CBS 8075. Ballistoconidia on corn meal agar. Bar = 5 µm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 30°C	+
Starch formation	–	Growth at 37°C	–
Urease	+		

Co-Q: 10 (Nakase 1989).
Mol% G + C: 56 (Nakase 1989).

Origin of strain studied: CBS 8075 (ATCC 52909, JCM 5355), leaf of *Banksia collina,* J.F. Brown, Australia.

Type strain: CBS 8075.

106.5. *Sporobolomyces gracilis* Derx (1930)

Growth on 5% malt extract agar: After 5 days at 17°C, the cells are cylindrical or ellipsoidal, (5.5–15.0) × (3.0–5.5) µm, and single. Budding is polar or lateral with buds sessile or on short denticles and with sympodial or percurrent proliferation. Colonies are butyrous, smooth or somewhat warty, shiny or dull, red (brick red), and with the margin entire, straight or crenulate.

Growth on the surface of assimilation media: Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, hyphae or pseudohyphae are absent. CBS 7028 forms short hyphal outgrowths on corn meal agar. Aerobic growth is red, shiny, butyrous, smooth, flat or with the center somewhat raised, and the margin entire.

Formation of ballistoconidia: Ballistoconidia were not observed. According to Derx (1930), they are pyriform, nearly symmetrical, (8.0–10.5) × (3.0–4.0) µm.

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	–
50% (w/w) Glucose	–	Growth at 25°C	v
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1986d).
Mol% G + C: 50.9 (Nakase 1989).

Origin of the strains studied: CBS 71 (ATCC 24258, IFO 1033, JCM 2936, NRRL Y-5504, VKM Y-677, NI 7598, NI 7615), rotten leaves, H.G. Derx; CBS 7028 (UCD-FST 78-52), M. Miranda; CBS 7123, leaf of *Phertinia glabra,* K. Wickremasinghe, Australia.

Type strain: CBS 71.

Comments: *Sporobolomyces gracilis* is characterized by its inabilities to assimilate galactose, L-sorbose, sucrose, nitrate and nitrite. Since ballistoconidium formation is now absent in the strains studied, *S. gracilis* may be confused with species of *Rhodotorula.* Physiologically similar *Rhodotorula* species are *R. aurantiaca, R. minuta* and *R. mucilaginosa. Rhodotorula aurantiaca* differs by assimilation of nitrate and nitrite; *R. minuta* can be differentiated by growth on L-arabinose, sucrose and melezitose, whereas *R. mucilaginosa* differs by growth on sucrose and raffinose.

106.6. *Sporobolomyces griseoflavus* Nakase & M. Suzuki (1987b)

Growth on 5% malt extract agar: After 5 days

at 25°C, the cells are ellipsoidal to fusiform, (7.5–10.0)×(2.0–3.5) μm, and single. Budding is polar, with buds sessile or on short denticles and with sympodial proliferation. Colonies are butyrous to mucoid, smooth, shiny, grayish-white to yellowish-cream, and with the margin entire and finely crenulate.

Growth on assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, no hyphae or pseudohyphae are formed. Aerobic growth is pale yellowish-brown, shiny, butyrous or mucoid, smooth, flat or with the center somewhat raised, and the margin entire.

Formation of ballistoconidia: Formation of ballistoconidia is poor. Ballistoconidia are allantoid to falcate, (5.0–6.0)×(2.0) μm. According to Nakase and Suzuki (1987b) they are abundantly formed, kidney- to sickle-shaped, and (4.5–8.5)×(2.0–3.5) μm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1987b).
Mol% G+C: 60.8–60.9, JCM 5653 (T_m: Nakase and Suzuki 1987b).

Origin of the strain studied: CBS 7284 (JCM 5653), dead leaves of *Sasa* species, T. Nakase, Japan.

Type strain: JCM 5653.

Comments: *Sporobolomyces griseoflavus* belongs to the "*singularis*" group, which is characterized by pale colonies and assimilation of 2-keto-D-gluconate. Among species belonging to this group, *S. griseoflavus* can be differentiated by growth on galactose, D-xylose, melezitose and starch, *S. falcatus* differs by lack of growth on D-arabinose, sucrose, maltose, melezitose and starch. Non-ballistoconidium forming species with a similar physiology are *Cryptococcus albidus*, *Rhodotorula aurantiaca*, *R. glutinis* and *R. pilati*. *Cryptococcus albidus* differs by growth on L-arabinose, and the *Rhodotorula* species do not assimilate starch.

106.7. *Sporobolomyces inositophilus* Nakase & M. Suzuki (1987e)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal to fusiform, (6.0–14.0)×(2.0–3.0) μm, and single. Cells sometimes form hyphal outgrowths. Budding is polar with buds sessile or on short denticles that show sympodial proliferation. Colonies are mucoid, smooth, shiny, grayish-cream, and with the margin entire, straight or somewhat undulate.

Growth on the surface of assimilation media: Islets and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, no hyphae or pseudohyphae are formed. On corn meal agar and malt extract agar hyphal outgrowths of up to 50 μm are present. Aerobic growth is whitish to yellowish-cream, shiny, butyrous, smooth, slightly striate, with the center somewhat raised, and with the margin entire.

Formation of ballistoconidia: Ballistoconidia are formed on corn meal agar and are reniform to falcate, (6.0–9.0)×(1.0–3.0) μm (Fig. 418).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	+
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

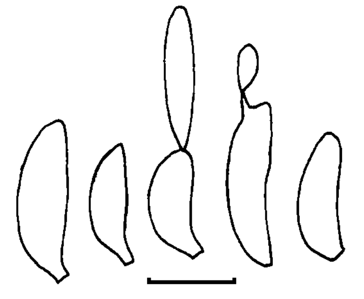


Fig. 418. *S. inositophilus*, CBS 7310. Ballistoconidia on corn meal agar. Bar = 5 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1987e).
Mol% G+C: 58.1, JCM 5654 (T_m: Nakase and Suzuki 1987e).

Origin of the strain studied: CBS 7310 (JCM 5654), dead leaf of *Sasa* species, T. Nakase, Japan.
Type strain: JCM 5654.

Comments: *Sporobolomyces inositophilus* belongs to the "*singularis*" group. The species can be differentiated from other species by assimilation of inositol.

106.8. *Sporobolomyces kluyveri-nielii* van der Walt (van der Walt et al. 1986a)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal, (8.5–10.0)×(4.0–6.5) μm, and single. Budding is polar, with buds sessile or on short denticles and with percurrent and sympodial proliferation. Colonies are butyrous, smooth, dull, brownish-orange, and with the margin entire, straight or undulate.

Growth on the surface of assimilation media (glucose): Islets and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, no hyphae or pseudohyphae are formed. On corn meal agar, irregularly branched hyphae occur. Aerobic growth is orange, dull, butyrous, slightly warty to low pustulate, with the center somewhat raised, and the margin entire, straight or crenulate.

Formation of ballistoconidia: Ballistoconidia occur on corn meal agar. They are allantoid or reniform, (10.0–20.0)×(4.0–8.5) μm (Fig. 419).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

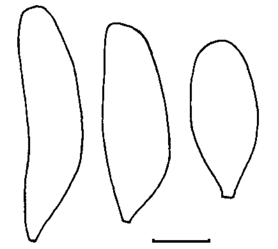


Fig. 419. *S. kluyveri-nielii*, CBS 7168. Ballistoconidia on corn meal agar. Bar = 5 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase 1989).
Mol% G+C: 53.8, CBS 7168 (T_m: van der Walt et al. 1986a).

Origin of the strain studied: CBS 7168, leaves of *Dombeya rotundifolia* J.P. van der Walt, South Africa.
Type strain: CBS 7168.

Comments: *Sporobolomyces kluyveri-nielii* can be identified by an orange colony color, no assimilation of trehalose, cellobiose, and by formation of large ballistoconidia.

106.9. *Sporobolomyces lactophilus* Nakase, M. Itoh, M. Suzuki & Bandoni (1990b)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal, allantoid or cylindroidal, (8.5–22.0)×(2.0–3.5) μm, and single. Budding is polar or lateral, with buds sessile, on denticles or on elongated stalks and showing sympodial proliferation. Colonies are butyrous or rather firm, flat, somewhat warty or reticulate, dull, cream, with the margin entire and crenulate.

Growth on the surface of assimilation media: Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, poorly developed pseudohyphae and short hyphal elements are present. Aerobic growth is yellowish-cream, dull, butyrous to rather firm, smooth, ridged, slightly warty or reticulate, flat or with the center somewhat raised, and with the margin entire and crenulate.

Formation of ballistoconidia: Ballistoconidia occur on corn meal agar. They are ellipsoidal, amygdaliform or falcate, (5.5–10.0)×(1.0–4.0) μm (Fig. 420).

Fermentation: absent.

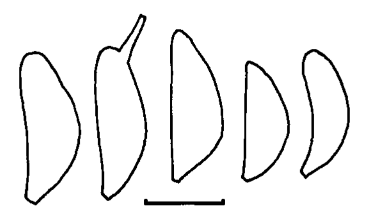


Fig. 420. *S. lactophilus*, CBS 7527. Ballistoconidia on corn meal agar. Bar = 5 μm.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% (w/w) Glucose	–	Growth at 30°C	+
Starch formation	–	Growth at 35°C	–
Urease	+		

Co-Q: 10 (Nakase et al. 1990b).
Mol% G + C: 56.3–57.1, two strains, JCM 7595, JCM 7596 (T_m: Nakase et al. 1990b); 53.8–55.3, two strains, JCM 7595, JCM 7596 (HPLC: Nakase et al. 1990b).

Origin of the strains studied: CBS 7527 (JCM 7595), JCM 7594, JCM 7597, all from dead branches of *Abies firma,* T. Nakase, Japan.
Type strain: JCM 7595.

Comments: Conidiogenesis on long stalks as it occurs in *S. lactophilus* is similar to that described for the genus *Ballistosporomyces* (Nakase et al. 1989b). Among the species with pale colonies and lack of growth with 2-keto-D-gluconate, *S. lactophilus* can be differentiated by absence of growth on raffinose and presence of growth on butanediol-2,3 (Boekhout 1991a).

106.10. *Sporobolomyces oryzicola* Nakase & M. Suzuki (1986b)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal or cylindroidal, (7.0–9.0)×(3.0–5.0) µm, and single. Budding is polar, with buds sessile and with percurrent proliferation. Colonies are butyrous to mucoid, smooth, shiny, salmon-red, and with the margin entire.

Growth on the surface of assimilation media (glucose): A ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, hyphae or pseudohyphae are absent. Aerobic growth is dark red, shiny, butyrous, smooth, and with the center somewhat raised and the margin entire.

Formation of ballistoconidia: Ballistoconidia occur on corn meal agar. They are ellipsoidal, (10.0–13.5)×(4.0–5.0) µm (Fig. 421).

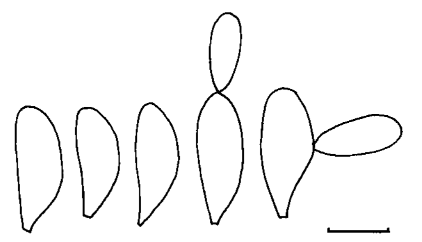


Fig. 421. *S. oryzicola,* CBS 7228. Ballistoconidia on corn meal agar. Bar = 5 µm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% (w/w) Glucose	–	Growth at 30°C	+
Starch formation	–	Growth at 37°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1986b).
Mol% G + C: 61.1, JCM 5299 (T_m: Nakase and Suzuki 1986b).

Origin of the strain studied: CBS 7228 (IFO 10180, JCM 5299), dead leaf of rice (*Oryza sativa*), T. Nakase, Japan.
Type strain: JCM 5299.

Comments: *Sporobolomyces oryzicola* is physiologically rather similar to *S. alborubescens.* This latter species can be differentiated by a slightly higher mol% G + C (61 vs. 63), growth on ethanol, and lack of growth on α-methyl-D-glucoside.

106.11. *Sporobolomyces phyllomatis* van der Walt & Y. Yamada (van der Walt et al. 1988)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal, ovoidal or cylindroidal, (8.0–16.0)×(3.0–7.0) µm, and single, or in short chains. Budding is polar, with buds sessile and with percurrent proliferation. Scars are distinct. Cells occasionally develop hyphae of up to ca. 55 µm long. Colonies are butyrous, smooth, shiny, orange-brown or pinkish-orange, and with the margin entire.

Growth on the surface of assimilation media: A ring and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, short hyphae and pseudohyphae occur (Fig. 422A). Aerobic growth is orange, brownish-orange or pinkish-orange, dull or shiny, smooth or slightly striate, flat or with the center somewhat raised, and with the margin entire.

Formation of ballistoconidia: Ballistoconidia are formed on corn meal agar. They are ellipsoidal to allantoidal, (7.0–13.0)×(3.0–5.0) μm (Fig. 422B).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

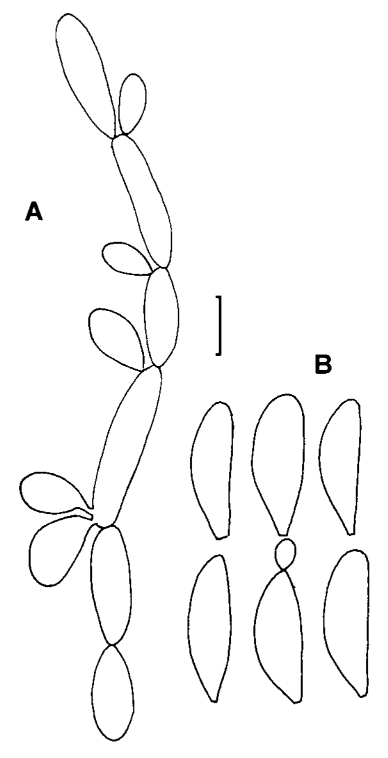


Fig. 422. *S. phyllomatis*, CBS 7198. (A) Pseudomycelium on morphology agar. (B) Ballistoconidia on corn meal agar. Bar = 5 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (van der Walt et al. 1988).

Mol% G + C: 47.4, CBS 7198 (T_m: van der Walt et al. 1988).

Origin of the strains studied: CBS 7198 (JCM 7549), CBS 7199, both from leaf of *Dombeya rotundifolia*, J.P. van der Walt, South Africa.

Type strain: CBS 7198.

Comments: *Sporobolomyces phyllomatis* shares with *S. kluyveri-nielii* and *S. salicinus* the characteristics of orange colonies and large ballistoconidia. *Sporobolomyces phyllomatis* can be differentiated from *S. kluyveri-nielii* by growth on L-rhamnose, sucrose, maltose, trehalose and melezitose, and lack of growth on nitrate and nitrite. *Sporobolomyces salicinus* differs by lack of growth on L-rhamnose, maltose, melezitose, 2-keto-D-gluconate, D-gluconate, and growth on nitrate and nitrite.

106.12. *Sporobolomyces roseus* Kluyver & van Niel (1924)

Synonyms:

?*Torula cinnabarina* Jörgensen (1909)
Sporobolomyces tenuis Kluyver & van Niel (1924)
Cryptococcus pulverulentus Beyerinck (nom. nud.) ex Kluyver and van Niel (1924)
Torula photographa Biourge (nom. nud.) ex Kluyver and van Niel (1924)
Sporobolomyces photographus (Biourge) Ciferri & Redaelli (1925)
Amphierna rubra Grüss (1926)
Sporobolomyces salmoneus Derx var. *salmoneus* Derx (1930)
Sporobolomyces salmonicolor (Fischer & Brebeck) Kluyver & van Niel var. *salmoneus* (Derx) Verona & Ciferri (1938)
Sporobolomyces salmoneus Derx var. *albus* Derx (1930)
Sporobolomyces salmonicolor (Fischer & Brebeck) Kluyver & van Niel var. *albus* (Derx) Verona & Ciferri (1938)
Torulopsis somala Verona (1935)
Sporobolomyces pollaccii Verona & Ciferri (1938)
Sporobolomyces miniatis Yamasaki & Fujii (1950) nom. illeg.
Sporobolomyces ruberrimus Yamasaki & Fujii (1950) var. *ruberrimus* nom. illeg.
Sporobolomyces ruberrimus Yamasaki & Fujii (1950) var. *albus* Yamasaki & Fujii (1950) nom. illeg.
Sporobolomyces roseus Kluyver & van Niel var. *madurae* A. Janke (1954)
Sporobolomyces boleticola C. Ramírez Gómez (1957)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal to cylindroidal, (9.0–24.0)×(3.0–8.5) μm, and single. Budding is usually polar, with buds sessile or on short denticles and with percurrent or sympodial proliferation. Cells sometimes germinate with slender hyphae. Hyphae and pseudohyphae are sometimes present. Colonies are butyrous or tending to mucoid, smooth, warty, venose to reticulate, sometimes striate, flat to raised, shiny or dull, orange to salmon-pink, and with the margin eroded, or entire and crenulate.

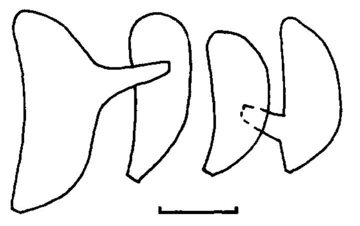


Fig. 423. *S. roseus*, CBS 485. Ballistoconidia on morphology agar. Bar = 5 µm.

Growth on the surface of assimilation media (glucose): A ring, a film and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, hyphae and pseudohyphae sometimes occur. Large globose to subglobose cells frequently occur, (8.0–25.0)×(7.0–16.0) µm. Aerobic growth is pinkish-red, orange-red or brownish-orange, dull or shiny, butyrous, smooth, reticulate or warty, flat, and with the margin eroded or entire.

Formation of ballistoconidia: Ballistoconidia are formed on many artificial media. They are ellipsoidal or allantoid, (7.0–14.0)×(3.0–6.0) µm (Fig. 423). Sterigmata frequently are sympodially branched.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	v	Glycerol	v
Maltose	+	Erythritol	–
Cellobiose	v	Ribitol	v
Trehalose	v	Galactitol	v
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	v	Salicin	v
Inulin	–	D-Gluconate	v
Soluble starch	v	DL-Lactate	v
D-Xylose	v	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	v
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase and Suzuki 1986d).

Mol% G+C: 50 (BD: Storck 1966); 53.5–56 (BD: Storck et al. 1969); 50.5–56.0 (Nakase 1989); 49.5–52.1, 6 strains (T_m: Boekhout 1991a).

Origin of the strains studied: CBS 485 (IFO 1037, NRRL Y-5480, NI 7593), mycotic lesion of mucous membranes, G. Pollacci, Italy; CBS 486 (ATCC 24257, JCM 5353, MUCL 30251, NRRL Y-5506, UCD-FST 68-353), M.W. Beyerinck; CBS 488 (IFO 1105, NRRL Y-5482, VKM Y-683), etiolated grass, H.G. Derx, Netherlands; CBS 492 (IFO 1040, NRRL Y-5485, NI 7601), diseased potatoes, M.W. Beyerinck, Netherlands; CBS 493 (NRRL Y-5477, VKM Y-686), nectar of *Linaria* sp., J. Grüss, Germany; CBS 494 (NRRL Y-5486), air, S.L. Schouten, Netherlands; CBS 993, soil, O. Verona, Somalia; CBS 2641, air, J.H. Becking, Netherlands; CBS 1036, wood pulp, P. Redaelli; CBS 2646, madura foot, A. Janke, Austria; CBS 5004, J. Ruinen, Indonesia; CBS 7500, A. Shiraishi, Japan; CBS 7501, pale mutant of CBS 7500.

Type strain: CBS 486.

Comments: *Sporobolomyces roseus* agrees in many aspects with *S. shibatanus*, the anamorph of *Sporidiobolus pararoseus*. They agree in the color of the colony, mol% G+C, partial 18S rRNA sequences (Takematsu et al. 1990), and many physiological test results. Mating experiments among isolates of *S. roseus* were negative (Boekhout 1991a). From a practical point of view, *S. roseus* can be differentiated from *S. shibatanus* by growth with nitrate and nitrite.

Sporobolomyces roseus occurs in the phyllosphere (Derx 1930, Tubaki 1953, Last 1955, van der Burg 1974). According to the latter author, *S. roseus* belongs to the so-called "sugar" fungi, which use honey-dew for growth.

106.13. *Sporobolomyces ruber* (Nakase, Okada & Sugiyama) Boekhout (1991a)

Synonym:
Ballistosporomyces ruber Nakase, Okada & Sugiyama (1989b)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal to fusiform, (6.0–9.0)×(2.0–3.0) µm, and single or in short branched chains. Budding is polar and lateral, with the buds usually on short denticles or stalks, which generally proliferate sympodially. Colonies are butyrous or rather tough, somewhat pustulate, dull or somewhat shiny, pinkish-red, and with the margin entire.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, hyphae and pseudohyphae are absent. Aerobic growth is orange-cream, orange-pink (salmon) or brownish-orange, dull or somewhat shiny, moist or somewhat tough, somewhat pustulate or reticulate, convex, and with the margin entire and crenulate.

Formation of ballistoconidia: Ballistoconidia occur on malt extract agar and potato-dextrose agar. They are allantoid, and measure (6.0–12.5)×(1.5–5.0) µm (Fig. 424).

Fermentation: absent.

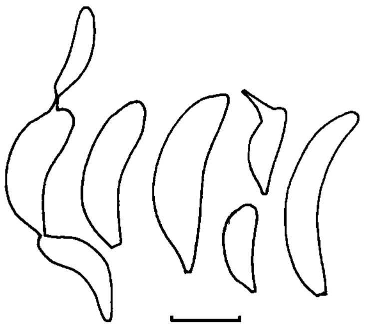


Fig. 424. *S. ruber,* CBS 7512. Ballistoconidia on malt extract agar. Bar = 5 µm.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	w/–	Nitrite	–
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	–		

Co-Q: 10 (Nakase et al. 1989b).
Mol% G+C: 50.8, JCM 6884 (T_m: Nakase et al. 1989b); 48.3, JCM 6884 (HPLC: Nakase et al. 1989b).

Origin of the strain studied: CBS 7512 (JCM 6884, NB 258), dead leaf of *Vitis ficifolia* var. *lobata,* T. Nakase, Japan.

Type strain: JCM 6884.

Comments: *Sporobolomyces ruber* has a distinctive mode of conidiogenesis in which conidia are usually formed on sympodially or percurrently proliferating denticles and stalks. Besides typical ballistoconidia, morphologically similar, but not actively discharged cells are formed. These types of reproduction were also observed in some other species, e.g., *Bensingtonia ingoldii, B. miscanthi* and *S. lactophilus* (Boekhout 1991a). *Sporobolomyces ruber* is characterized by salmon-orange colonies, lack of growth on trehalose and by its mode of conidiogenesis.

106.14. *Sporobolomyces salicinus* (Johri & Bandoni) Nakase & M. Itoh (1988)

Synonym:
Bullera salicina Johri & Bandoni (1984)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal, cylindrical or subglobose, (8.0–14.0)×(4.0–7.0) µm, and single. Budding is polar, with the buds sessile or on short denticles and showing percurrent or sympodial proliferation. Scars are present. Pseudohyphae and short hyphae occasionally occur. Colonies are butyrous, smooth or slightly warty, dull or shiny, orange or brownish-orange, and with the margin entire, straight or crenulate.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, poorly developed pseudohyphae occur. Aerobic growth is pinkish to brownish-orange, shiny or dull, butyrous, smooth or somewhat warty, flat, and with the margin entire and straight.

Formation of ballistoconidia: Ballistoconidia occur on corn meal agar. They are ellipsoidal to allantoid, and measure (12.0–23.0)×(5.0–8.0) µm (Fig. 425).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

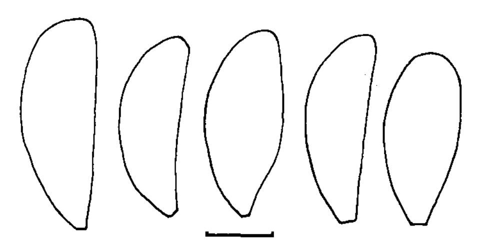


Fig. 425. *S. salicinus,* CBS 6983. Ballistoconidia on corn meal agar. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% (w/w) Glucose	–	Growth at 30°C	+
Starch formation	–	Growth at 37°C	–
Urease	+		

Co-Q: 10 (Nakase 1989).
Mol% G+C: 51.1, JCM 2959 (T_m: Nakase and Itoh 1988).

Origin of the strain studied: CBS 6983 (JCM 2959, UBC 8081), leaf of willow (*Salix* sp.), R.J. Bandoni, British Colombia, Canada.

Type strain: UBC 8081.

Comments: *Sporobolomyces salicinus* has orange colonies and large ballistoconidia. These characteristics also occur in *S. kluyveri-nielii* and *S. phyllomatis*. *Sporobolomyces salicinus* can be differentiated from these species by growth on salicin and raffinose, and lack of growth on 2-keto-D-gluconate and D-gluconate. According to Takematsu et al. (1990), partial 18S rRNA sequences of *S. salicinus* are similar to those of *S. phyllomatis* and *S. gracilis*. Enteroblastic conidiation, scars and a thickened cell wall are obvious in older cultures.

106.15. *Sporobolomyces salmonicolor* (Fischer & Brebeck) Kluyver & van Niel (1924)

See under teleomorph *Sporidiobolus johnsonii* Nyland, p. 694

106.16. *Sporobolomyces sasicola* Nakase & M. Suzuki (1987b)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal or cylindroidal, (7.0–15.0)×(2.0–3.5) µm, and single. Budding is usually polar and sessile. Colonies are butyrous, smooth or somewhat warty, shiny, yellowish-cream, and with the margin entire and straight.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, hyphae and pseudohyphae are absent. Aerobic growth is pale yellowish-brown, shiny or dull, butyrous, flat, ridged or somewhat reticulate, slightly inflated toward the margin, and with the margin entire, straight, undulate or crenulate.

Formation of ballistoconidia: Ballistoconidia occur on corn meal agar, malt extract agar and potato-dextrose agar. They are allantoid, (6.0–10.0)×(2.0–3.0) µm (Fig. 426).

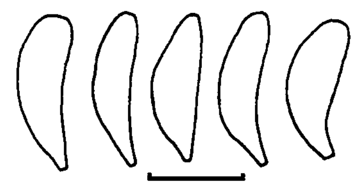


Fig. 426. *S. sasicola*, CBS 7285. Ballistoconidia on malt extract agar. Bar = 5 µm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	–
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1987b).
Mol% G+C: 56.9, JCM 5979 (T_m: Nakase and Suzuki 1987b).

Origin of the strain studied: CBS 7285 (JCM 5979), dead leaf of *Sasa* sp., T. Nakase, Japan.

Type strain: JCM 5979.

Comments: *Sporobolomyces sasicola* is characterized by pale colonies and lack of growth with 2-keto-D-gluconate. It differs from other species with these characteristics, viz. *S. lactophilus, S. subbrunneus* and *S. xanthus,* by growth with L-arabinose, and lack of growth with citrate, nitrate and nitrite.

106.17. *Sporobolomyces shibatanus* (Okunuki) Verona & Ciferri (1938)

See under teleomorph *Sporidiobolus pararoseus* Fell & Tallman, p. 694

106.18. *Sporobolomyces singularis* Phaff & do Carmo-Sousa (1962)

Synonym:
Bullera singularis (Phaff & do Carmo-Sousa) Rodrigues de Miranda (1984c)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal to subglobose, (6.0–12.0)×(2.5–6.0) µm, and single. Budding is polar, with the buds sessile or on short denticles and with percurrent or sympodial proliferation. Colonies are mucoid, smooth, shiny, pale yellowish-brown, and with the margin entire and straight.

Growth on the surface of assimilation media: Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After

7 days at 25°C, no hyphae or pseudohyphae are formed. Aerobic growth is whitish to cream, shiny, mucoid, smooth, somewhat raised, and with the margin entire.

Formation of ballistoconidia: Ballistoconidia were not observed. According to Phaff and do Carmo-Sousa (1962), they are bilaterally symmetrical, and kidney- to sickle-shaped.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	–
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase 1989).
Mol% G+C: 58.0 (Nakase 1989).

Origin of the strain studied: CBS 5109 (ATCC 24193, JCM 5356, NCYC 900, NRRL Y-5852, UCD-FST 60-79), frass from *Scolytus tsugae* in dead specimen of *Tsuga heterophylla*, H.J. Phaff and L. do Carmo-Sousa, Oregon, USA.

Type strain: UCD-FST 60-79 (CBS 5109).

Comments: *Sporobolomyces singularis* belongs to a group of species with pale colonies and that assimilate 2-keto-D-gluconate. *S. singularis* can be differentiated from other species of this group by a combination of mucoid colonies and lack of assimilation of D-xylose, sucrose, maltose, nitrate and nitrite. *Rhodotorula foliorum* and *R. lignophila* do not form ballistoconidia but are physiologically and morphologically similar. *Rhodotorula foliorum* differs by assimilation of arbutin, nitrate and nitrite, while *R. lignophila* fails to grow with D-arabinose, salicin and ribitol.

106.19. *Sporobolomyces subbrunneus* Nakase & M. Suzuki (1985c)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are fusiform to ellipsoidal, (7.5–11.0)×(2.0–3.0) µm, and single. Budding is polar with the buds usually sessile. After prolonged cultivation poorly developed pseudohyphae sometimes occur. Colonies are butyrous, smooth to slightly warty, dull, pale yellowish-brown, and with the margin entire and straight.

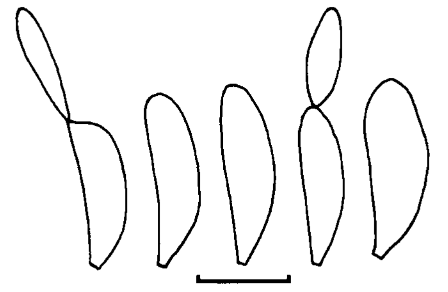


Fig. 427. *S. subbrunneus*, CBS 7196. Ballistoconidia on malt extract agar. Bar = 5 µm.

Growth on the surface of assimilation media (glucose): Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, no hyphae or pseudohyphae occur. Aerobic growth is pale yellowish-brown, dull, butyrous, smooth or slightly warty, flat, and with the margin entire.

Formation of ballistoconidia: Ballistoconidia occur on malt extract agar and yeast extract–malt extract agar. They are ellipsoidal or allantoid, (6.0–11.0)×(2.5-4.0) µm (Fig. 427).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10 (Nakase and Suzuki 1985c).
Mol% G+C: 68, JCM 5278 (T_m: Nakase and Suzuki 1985c).

Origin of the strain studied: CBS 7196 (IFO 10168, JCM 5278), rice (*Oryza sativa*), T. Nakase, Japan.

Type strain: JCM 5278.

Comments: *Sporobolomyces subbrunneus* belongs to

a group of species with pale colonies and inability to assimilate 2-keto-D-gluconate. Among these species, *S. subbrunneus* can be characterized by its lack of growth with maltose, cellobiose, and by assimilation of lactate and D-gluconate.

106.20. *Sporobolomyces tsugae* (Phaff & do Carmo-Sousa) Nakase & M. Itoh (1988)

Synonym:
Bullera tsugae Phaff & do Carmo-Sousa (1962)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are globose to subglobose or ellipsoidal, (3.5–8.0)×(5.0–7.5) μm, and single. Short germ hyphae sometimes occur. Budding is polar, with percurrent or sympodial proliferation. Colonies are mucoid, smooth, shiny, cream to pale yellowish-brown, lack color near the margin, and with an entire, straight or undulate margin.

Growth on the surface of assimilation media (glucose): A ring, islets and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, no hyphae or pseudohyphae are formed. Aerobic growth is whitish to cream, lacking color near the margin, shiny, mucoid, smooth, convex, and with the margin entire, straight or undulate.

Formation of ballistoconidia: Ballistoconidia are formed on morphology agar. They are ellipsoid, (6.0–10.5)×(3.0–7.0) μm (Fig. 428).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	–	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

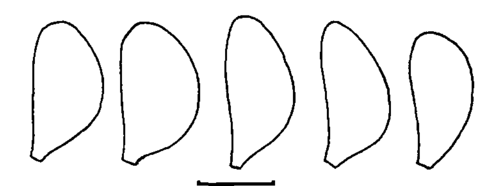


Fig. 428. *S. tsugae*, CBS 7096. Ballistoconidia on morphology agar. Bar = 5 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	v
Urease	+	Growth at 37°C	–

Co-Q: 10 (Nakase 1989).
Mol% G+C: 50.4–52.3, two strains, CBS 5038, CBS 7096 (T_m: Boekhout 1991a); 50.4 (Nakase 1989).

Origin of the strains studied: CBS 5038 (ATCC 18802, JCM 2960, UCD-FST 60-71), frass from an insect under the bark of *Tsuga heterophylla*, H.J. Phaff and L. do Carmo-Sousa, Oregon, USA; CBS 7096, polluted water, F. Hinzelin, France; ICG 4441, rotten trunk of a tree, A. Fonseca, Portugal.

Type strain: UCD-FST 60-71 (CBS 5038).

Comments: *Sporobolomyces tsugae* belongs to a group of species with pale colonies that assimilate 2-keto-D-gluconate. Among these species, *S. tsugae* can be differentiated by assimilation of L-sorbose and α-methyl-D-glucoside. According to Phaff and do Carmo-Sousa (1962), ballistoconidia are globose (UCD-FST 60-71). However, no ballistoconidia were observed in this strain by the present authors. CBS 7096 and ICG 4441 form distinct bilaterally symmetrical ballistoconidia.

106.21. *Sporobolomyces xanthus* (Nakase, Okada & Sugiyama) Boekhout (1991a)

Synonym:
Ballistosporomyces xanthus Nakase, Okada & Sugiyama (1989b)

Growth on 5% malt extract agar: After 5 days at 25°C, the cells are ellipsoidal or allantoid, (7.0–10.0)×(2.5–4.0) μm, and single. Budding is multipolar, usually on short denticles, and with the buds frequently allantoid. Colonies are butyrous, smooth, shiny, pale yellowish-brown, and with the margin entire and straight.

Growth on the surface of assimilation media: Absent, but a sediment is formed.

Dalmau plate culture on morphology agar: After 7 days at 25°C, hyphae and pseudohyphae are absent, but yeast cells form undifferentiated chains. Aerobic growth is yellow-brown or orange-yellow, shiny, but towards the margin becoming dull, butyrous, flat, somewhat reticulate, near the margin slightly striate, and with the margin entire, straight or lobate.

Formation of ballistoconidia: Ballistoconidia occur on corn meal agar and malt extract agar. They are allantoid, (4.5–9.0)×(2.0–4.0) μm (Fig. 429).

Fermentation: absent.

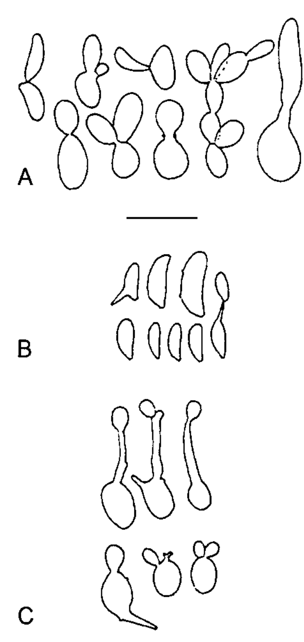


Fig. 429. *S. xanthus*, CBS 7513. (A) Yeast cells on morphology agar. (B) Ballistoconidia on corn meal agar. (C) Formation of stalked conidia on yeast extract–malt extract agar. Bar = 10 μm.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	–
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	+	DL-Lactate	–
D-Xylose	–	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% (w/w) Glucose	–	Growth at 30°C	+
Starch formation	–	Growth at 37°C	–
Urease	+		

Co-Q: 10 (Nakase et al. 1989b).

Mol% G + C: 62.1, JCM 6885 (T_m: Nakase et al. 1989b); 56.8, JCM 6885 (HPLC: Nakase et al. 1989b).

Origin of the strains studied: CBS 7513 (JCM 6885, NB 206), dead leaf of *Acer rufinerve,* T. Nakase, Japan.

Type strain: JCM 6585.

Comments: *Sporobolomyces xanthus* belongs to a group of species with pale colonies and which do not grow with 2-keto-D-gluconate. Among the species with these characteristics, *S. xanthus* can be differentiated by growth on D-galactose and from its morphology.

Comments on the genus

The genus *Sporobolomyces* is characterized by the formation of ballistoconidia, lack of xylose in whole-cell hydrolyzates, and, usually, Co-Q 10 as the major ubiquinone. Several species have red, orange or pink colonies due the presence of β-carotenoids, γ-carotenoids, torulene and torularhodine (Valadon 1966, Fiasson 1967, Fiasson et al. 1973). Recently, species with pale colonies have been described, which, presumably, do not form these pigments. The pale colored species can be divided into two groups based on the assimilation of 2-keto-D-gluconate (Nakase 1989). A considerable heterogeneity of the genus is indicated by rRNA sequences (Takematsu et al. 1990, Fell et al. 1992). The presence of Co-Q 10(H_2) in *S. elongatus* (Nakase and Suzuki 1986d) suggests a further heterogeneity within the genus. Subdivision of the genus based on the presence or absence of pseudohyphae (Ciferri and Redaelli 1929, Novák and Zsolt 1961) was rejected by Lodder and Kreger-van Rij (1952).

Two species, viz. *S. salmonicolor* and *S. shibatanus,* have their teleomorphs in the teliospore-forming genus *Sporidiobolus.* Differences in DNA contents among cells of a strain of *S. salmonicolor* were interpreted to represent haploid and diploid stages (van der Walt and Pitout 1969). A teleomorph genus, *Aessosporon* van der Walt, with *A. salmonicolor* van der Walt as type species was described based on this supposedly diploid stage (van der Walt 1970f). However, later authors observed mating, including formation of dikaryotic hyphae with clamp connections and teliospores, between the type strains of *A. salmonicolor* and *S. salmonicolor* (Fell and Statzell-Tallman 1980b). Therefore, the genus *Aessosporon* is not recognized as representing the teleomorph.

A close taxonomic relationship has been proposed between species of *Sporobolomyces* and the genus *Rhodotorula* (Janke 1954, Crook and Johnston 1962, Tsuchiya et al. 1969, Weijman and Rodrigues de Miranda 1988, Golubev 1989a,b, Dörfler 1990). This is supported by nucleotide sequences of the large subunit rRNAs of species belonging to both genera, which resulted in heterogeneous dendrograms (Fell et al. 1992). This may indicate that currently used generic concepts are not adequate from an evolutionary point of view. However, for practical reasons, we maintain the generic separation of ballistoconidia-forming and non-ballistoconidia-forming genera.

Conidiogenesis is usually polar, but sometimes may also be multipolar. Cells are usually formed on short denticles, but in some species elongate denticles or stalks occur (Boekhout 1991a). Scars may be distinct (de Hoog 1982, Boekhout 1991a), and proliferation is percurrent and/or sympodial.

The genus *Ballistosporomyces* was described for two species, which were reported to reproduce by formation

of ballistoconidia and inactively discharged conidia, but not by budding cells (Nakase et al. 1989b). The inactively discharged conidia are formed on denticles or stalks, which proliferate sympodially or percurrently. However, these types of conidiogenesis were observed in other species of *Sporobolomyces* as well, e.g., in *S. lactophilus,* and budding cells do occur in the genus *Ballistosporomyces* (Boekhout 1991a). Therefore, *Ballistosporomyces* is considered to be congeneric with *Sporobolomyces* (Boekhout 1991a). Conidium formation on elongate denticles and stalks, as observed in some species of *Sporobolomyces* and *Bensingtonia* (Boekhout 1991a), and other characteristics, such as ubiquinone and carbohydrate composition, are similar to those characteristics in *Kurtzmanomyces* (Yamada et al. 1988a, Nakase et al. 1989b).

Several species of *Sporobolomyces,* e.g., *S. roseus,* widely occur in nature. An important habitat is the phyllosphere (Derx 1930). Air-borne propagules of *Sporobolomyces* can cause respiratory allergy (Evans 1965).

107. *Sterigmatomyces* Fell emend. Y. Yamada & Banno

I. Banno and Y. Yamada

Diagnosis of the genus

Cells are spherical to ovoidal and produce one or more short conidiogenous stalks (1.5–3.0 µm length). A conidium develops terminally on the stalk. When the new cell (conidium) is mature it may in turn produce stalks, often resulting in short branched chains of cells. Newly formed cells separate at a septum, in the mid-region of the stalk on the parent cell. True mycelium is not produced. Ballistoconidia are not produced.

Glucose is not fermented. Nitrate may or may not be assimilated. *myo*-Inositol is not assimilated. Diazonium blue B color reaction is positive. Xylose is absent in the cells. Coenzyme Q-9 is present.

Type species

Sterigmatomyces halophilus Fell

Species accepted

1. *Sterigmatomyces elviae* Sonck & Yarrow (1969)
2. *Sterigmatomyces halophilus* Fell (1966)

Key to species

See Table 93.

1. a Sucrose assimilated, growth at 37°C .... *S. elviae*: p. 844
   b. Sucrose not assimilated, growth absent at 37°C .... *S. halophilus*: p. 845

Table 93
Key characters of the species in the genus *Sterigmatomyces*

Species	Assimilation				Growth at 37°C	Mol% G+C
	Sucrose	Lactose	Raffinose	Nitrate		
Sterigmatomyceselviae	+	+	+	–	+	52.0
S. halophilus	–	v	–	v	–	55.3

Systematic discussion of the species

107.1. *Sterigmatomyces elviae* Sonck & Yarrow (1969)

Growth in malt extract: After 3 days at 24°C, the cells are spherical to ovoidal, (3–6)×(3–10) µm, and reproduce by the formation of conidia on one to five short (<2 µm) stalks on each cell. The conidia are separated at the mid-point of the stalks on the parent cell. Occasionally, the conidia form additional stalked conidia and appear as a chain of conidia. Conidiogenous stalks are often so short that cell propagation appears to be typical budding. A light ring and sediment are present. After one month the cells are within the same size range; a moderate ring and sediment are present.

Growth on malt agar: Two colony types are found; in one type the streak culture is smooth, dull, cream-colored, edges entire, with cells similar to those described in malt extract. The other type differs and forms a glistening and viscous colony. Cells are ellipsoidal and apiculate, (2–7)×(3–13) µm, with one, or rarely, more short stalks (0.5 µm) and the general appearance of a typically budding yeast cell.

Dalmau plate culture on corn meal agar: Pseudomycelium and true mycelium are absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	w/–
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	s/+
Soluble starch	–	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Urease	+
Arbutin	+	0.01% Cycloheximide	–
Ethylamine	v	Growth at 37°C	+
60% Glucose	+		

Co-Q: 9 (Yamada and Banno 1984b).

Mol% G+C: 52.8 (T_m), type strain (CBS 5922); 51.2 (HPLC), IFO 10197.

Xylose in cells: Absent.

Origin of the strains studied: IFO 1843 (CBS 5922), groin of a man with seborrheic eczema, C.E. Sonck; IFO 10196 (CBS 8119), air in bakery, D. Swinne; IFO 10197 (CBS 8121), flour, D. Swinne.

Type strain: CBS 5922.

107.2. *Sterigmatomyces halophilus* Fell (1966)

Synonyms:

Sterigmatomyces halophilus Fell var. *indicus* Fell (1966)
Sterigmatomyces indicus (Fell) Fell (1970a)

Growth in malt extract: After 3 days at room temperature, the cells are spherical to ovoidal, (2–7)×(3–11) µm, with one to ten short conidiogenous stalks, 0.5×(1–3) µm (Fig. 430). New cells (conidia) are formed on the stalks. The conidia can produce secondary stalked conidia with old cells, resulting in short branched chains. Cells are separated by septal formation in the mid-region of the stalks. A light film is produced which develops into powdery islets and after one month there is a heavy white pellicle. A sediment is present.

Growth on malt agar: The cell morphology is similar to that in malt extract. The cells are varied in size, (1–4)×(3–17) µm, and some are thin and long (127 µm). The stalks are generally short 0.5×(1–3) µm; some are elongated to 26 µm. The width of the elongated stalk remains about 0.5 µm. The streak is chalky-white, pasty with a dry granular surface, and the border is irregular to lobate. Colonies may develop that are smooth, glistening or dull and with an entire margin.

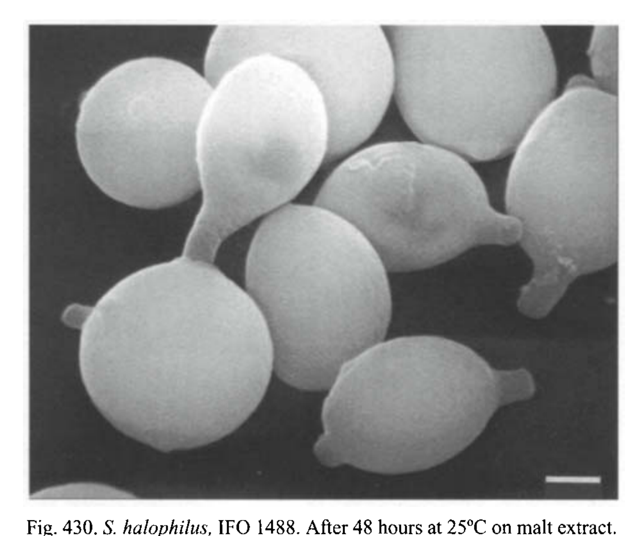


Fig. 430. *S. halophilus*, IFO 1488. After 48 hours at 25°C on malt extract. Scanning electron micrograph. Bar = 1 µm.

Dalmau plate culture on corn meal agar: Pseudomycelium and true mycelium are absent. Short branched chains of cells may be produced.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	w/–
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+/w
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	v	Ribitol	+
Trehalose	+	Galactitol	w/–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	+/w	Inositol	–
D-Ribose	+	Hexadecane	–
L-Rhamnose	–	Nitrate	v
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Starch formation	–
5-Keto-D-gluconate	+	Urease	+
Arbutin	+	0.01% Cycloheximide	–
Ethylamine	v	Growth at 25°C	+
60% Glucose	+	Growth at 30°C	–

Co-Q: 9 (Yamada and Banno 1984b).

Mol% G+C: 54.9–55.2, 4 strains (Fell et al. 1984a); 55.6 (HPLC), IFO 10201.

Xylose in cells: Absent.

Origin of the strains studied: IFO 1488 (CBS 4609), atmosphere, B. Ojeda; IFO 1844 (CBS 5628) type strain of *S. indicus*, Indian ocean, J.W. Fell, U.S.A.; IFO 10198 (CBS 5449), keloid blastomycosis, C. da Silva Lacaz; IFO 10119 (CBS 5632), sea water, J.W. Fell, U.S.A.; IFO 10200 (CBS 6780), soil, W. Gams, Netherlands; IFO 10201 (CBS 5629), sea water, J.W. Fell, U.S.A; IFO 10202 (CBS 5631), sea water, J.W. Fell, U.S.A.

Type strain: CBS 4609 (IFO 1488).

Comments on the genus

Vegetative cells produce short stalks terminally developing only one bud (conidium), which is separated at the mid-point of the stalk. The coenzyme Q system is mainly Co-Q 9. These characteristics clearly distinguish *Sterigmatomyces* from *Fellomyces* and *Kurtzmanomyces*. *S. indicus* was previously separated from *S. halophilus* on the basis of nitrate utilization. However, Kurtzman (in Fell et al. 1984a) and Kurtzman (1990a) considered that *S. indicus* is synonymous with *S. halophilus*, since the two species exhibited 100% DNA reassociation. Yamada et al. (1986a) supported this idea by a comparison of electrophoretic enzyme patterns.

108. *Sympodiomycopsis* Sugiyama, Tokuoka & Komagata

J. Sugiyama and S.-O. Suh

Diagnosis of the genus

Colonies on malt agar are dull cream-colored. Asexual reproduction is principally enteroblastic-annellidic and rarely holoblastic-sympodial. Vegetative cells are obclavate or obovoid and true mycelium is sometimes developed. Ascospores, teliospores, ballistospores, and sterigmatospores have not been observed.

Sugars are not fermented. The following tests are positive: extracellular DNase, urease and diazonium blue B. Xylose is present in trace amounts in the cells. The major ubiquinone system is Q-10.

Type species

Sympodiomycopsis paphiopedili Sugiyama, Tokuoka & Komagata

Species accepted

1. *Sympodiomycopsis paphiopedili* Sugiyama, Tokuoka & Komagata (1991)

Systematic discussion of the species

108.1. *Sympodiomycopsis paphiopedili* Sugiyama, Tokuoka & Komagata (1991)

Growth in 5% malt extract: After 3 days at 25°C, the cells vary from ovoid to elongate, sometimes subglobose, (4–11)×(2–6) μm, occurring singly, in pairs or in short chains, and reproducing principally by enteroblastic-annellidic, or rarely, by holoblastic-sympodial budding (Figs. 431, 432).

Growth on 5% malt extract agar: After 3 days at 25°C, the streak culture is dull and cream-colored; the surface is smooth, and colony texture is soft and slightly mucoid.

Dalmau plate culture on corn meal agar: After 14 days at 25°C, true mycelium is sometimes formed. Mycelium is composed of hyphae which are hyaline, smooth, thin-walled, septate, branched, 1–2 μm wide, rarely 5 μm wide, and often torulose. Ascospores, teliospores, ballistospores and sterigmatospores have not been observed.

Fermentation: absent.

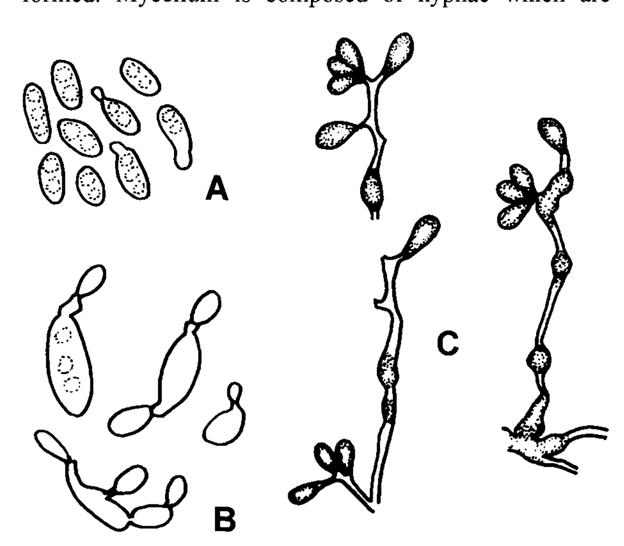


Fig. 431. *S. paphiopedili,* IAM 13459. (A)–(B) In malt extract after 4–7 days. (C) On corn meal agar after 4–25 days. Undifferentiated hyphae with terminal, intercalary or scattered conidiogenous cells; some of these bear 3 or more conidia in sympodial order.

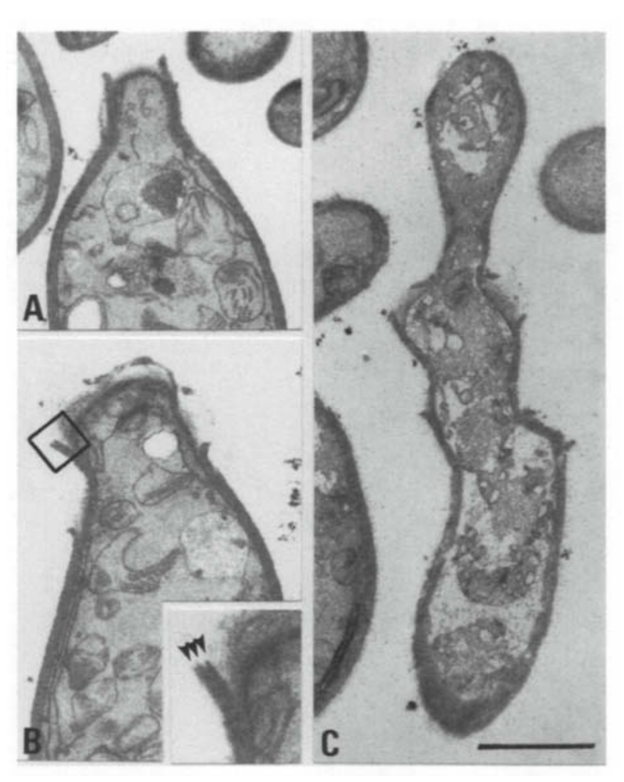


Fig. 432. *S. paphiodedili,* TEM micrographs. (A) Enteroblastic-annellidic conidiogenesis in young yeast cells. (B) Bud scars at the conidiogenous tip. Arrrows indicate multilayered cell wall of the parent cell. (C) Successive conidiogenesis occurs at the newly developed apex. Bar = 1 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	w	Methanol	n
L-Sorbose	+	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	w	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	–
Inulin	w	D-Gluconate	n
Soluble starch	w	DL-Lactate	w
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	–
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

Starch formation	–	Extracellular DNase	+
Urease	w	Growth at 37°C	–

Co-Q: 10 (Sugiyama et al. 1991).
Mol% G+C: 56.3 (T_m: Sugiyama et al. 1991).
Xylose in the cells: Present in trace amounts (Sugiyama et al. 1991).

Origin of the strain studied: Orchid (*Paphiopedilium primurinum*) nectar collected at the Koishikawa Botanic Garden of the University of Tokyo, Japan (Sugiyama et al. 1991).

Type strain: IAM 13459 (CBS 7429).

Comments on the genus

Sympodiomycopsis paphiopedili is usually dominant as a yeast morph, although it is associated with a hyphal morph. Sugiyama et al. (1991) considered that *S. paphiopedili* is basidiomycetous because of the following characteristics: positive reaction for urease, DNase and diazonium blue B color tests, Q-10 ubiquinone system, relatively high G+C content, and a basidiomycetous type of cell wall ultrastructure (Fig. 432B).

Morphologically, *S. paphiopedili* is similar to *Sympodiomyces parvus* Fell & Statzell (1971), which is characterized by a yeast morph and inconspicuous hyphal morph. The mode of conidiogenesis of these two genera is superficially similar. However, *Sympodiomyces parvus* is considered to be an ascomycetous yeast by van der Walt and Hopsu-Havu (1976) and Fell (1984b) because of negative urease and diazonium blue B color reactions, and a cell wall ultrastructure typical of ascomycetous yeasts. The septal pore ultrastructure of *S. paphiopedili* resembles that found in members of the Ustilaginales (Suh et al. 1993a). The 18S rDNA sequence-based tree also shows *S. paphiopedili* to be phylogenetically close to *Tilletia caries*, a smut fungus (Suh and Sugiyama 1994).

109. *Tilletiopsis* Derx ex Derx

T. Boekhout

Diagnosis of the genus

Hyphae are regularly branched, narrow, septate, hyaline, monokaryotic, usually partly lysed, and with retraction septa; septal pores are micropore-like. Ballistoconidia are bilaterally symmetrical, allantoid, falcate or cylindrical, and formed on sterigmata. Chlamydospores are sometimes present and hyaline or pigmented. Clamp connections are absent. Colonies are cream-colored, pinkish-cream, pinkish-yellow, yellow-brown or brown.

Diazonium blue B and urease reactions are positive. Xylose is absent from whole-cell hydrolyzates. Major ubiquinone is Q-10. Starch-like compounds are not produced.

Type species

Tilletiopsis washingtonensis Nyland

Species accepted

1. *Tilletiopsis albescens* Gokhale (1972)
2. *Tilletiopsis flava* (Tubaki) Boekhout (1991)
3. *Tilletiopsis fulvescens* Gokhale (1972)
4. *Tilletiopsis minor* Nyland (1950)
5. *Tilletiopsis pallescens* Gokhale (1972)
6. *Tilletiopsis washingtonensis* Nyland (1950)

Key to species

See Table 94.

1. a Inositol and α-methyl-D-glucoside assimilated; vitamins not required; colonies with considerable expansion growth → 2
   b Inositol and α-methyl-D-glucoside not assimilated; vitamins required; colonies with limited expansion growth → 3

2(1). a Colonies whitish, usually with hyaline droplets on the surface; mol% G+C 55–59 .... *T. albescens*: p. 848
   b Colonies whitish-cream to pale grayish-cream, without droplets on the surface; mol% G+C 42–46 .... *T. pallescens*: p. 851

3(1). a L-Sorbose assimilated; colonies pale yellow-brown, ochraceous or brown, firm → 4
   b L-Sorbose not assimilated; colonies pinkish, soft .... *T. washingtonensis*: p. 852

4(3). a Galactose and melezitose assimilated → 5
   b Galactose and melezitose not assimilated .... *T. flava*: p. 849

5(4). a Fusiform blastoconidia forming acropetal chains; mol% G+C 66–68 .... *T. fulvescens*: p. 850
   b Fusiform blastoconidia rarely forming acropetal chains; mol% G+C 55–57 .... *T. minor*: p. 851

Table 94
Key characters of species in the genus *Tilletiopsis*

Species	Assimilation						Growth in vitamin-free medium	Mol% G+C
	Galactose	L-Sorbose	Lactose	Melezitose	D-Gluconate	Inositol		
Tilletiopsis albescens	+	+	+	+	+	+	+	55–58.5(–63.5)
T. flava	–	+	–	–	–	–	–	63
T. fulvescens	+	v	+	+	v	–	–	66.5–68
T. minor	+	v	+	+	v	–	–	55–57
T. pallescens	v	+	v	+	v	v	+	42–45.5
T. washingtonensis	v	–	–	+	+	–	–	66.5–70

Systematic discussion of the species

109.1. *Tilletiopsis albescens* Gokhale (1972)

Growth on 5% malt extract agar: After 5 days at 17°C, hyphae are regularly branched, narrow, with cells measuring (10–55)×(1.5–3.5) μm, and with retraction septa (Fig. 433). Colonies are tough, flat, glabrous or pruinose, dull, whitish-cream, and with an eroded margin.

Growth on the surface of assimilation media: A ring and a thick film are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, hyphae are abundantly formed. Inflated cells with refractive contents occur and measure (20–40)×(3.0–6.0) µm. Chlamydospores occur intercalarily or terminally, are subglobose, ellipsoidal or cylindrical, and are solitary or catenate, (13–20)×(5.0–15.0) µm. Aerobic growth is whitish to yellowish-cream, dull, flat, frequently somewhat raised in the center and the margin eroded.

Formation of ballistoconidia: Ballistoconidia form on sterigmata and are allantoid, falcate or cylindrical, (7.0–16.0)×(1.0–4.0) µm (Fig. 433).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	+	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	w/–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

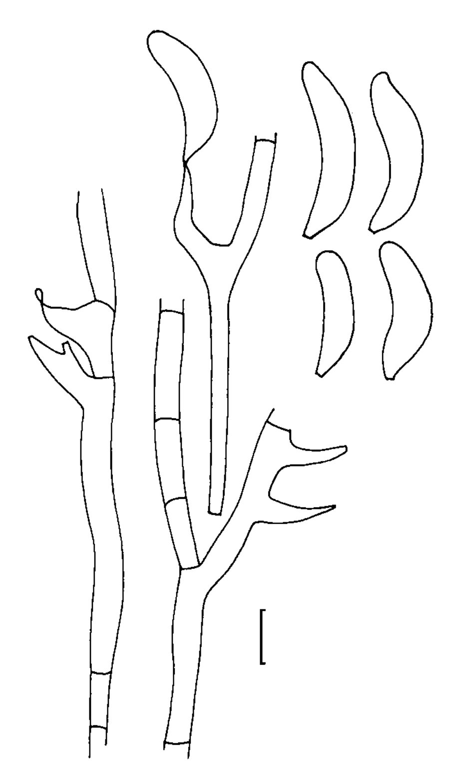


Fig. 433. *T. albescens*, CBS 608.83. Hyphae and ballistoconidia on malt extract agar. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Nitrite	+
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	–
Urease	+		

Co-Q: 10, CBS 608.83, CBS 370.85, (Boekhout et al. 1992a).
Mol% G + C: 55.0–58.5 (–63.5), 6 strains (T_m: Boekhout et al. 1992b).

Origin of the strains studied: CBS 608.83 (ATCC 24343, UBC 926, YK 801), sewage, W.B. Cooke, Cincinnati, Ohio, USA; CBS 370.85, barley powdery mildew culture, J.P. Skou, Denmark; CBS 361.86 (IFO 9015, YK 802), origin unknown, Japan; UAMH 5028, lesion of colostomy, I. Weitzman, New York, USA.

Type strain: UBC 926.

Comments: *Tilletiopsis albescens* is morphologically and physiologically similar to *T. pallescens*. It can be differentiated from this species by colonies that are whiter, and a higher mol% G + C of ca. 55–59. Yamazaki et al. (1985) observed low similarity between electrophoretic enzyme patterns of these species.

109.2. *Tilletiopsis flava* (Tubaki) Boekhout (1991a)

Synonym:
Tilletiopsis minor Nyland var. *flava* Tubaki (1952)

Growth on 5% malt extract agar: After 5 days at 17°C, hyphae are regularly branched, narrow, with cells measuring (15–100)×(1.5–2.0) µm, and with retraction septa (Fig. 434). Colonies are soft or somewhat tough, warty or reticulate, somewhat pruinose, dull, pale yellowish-brown, and with an entire margin.

Growth on the surface of assimilation media: A thin film is formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, hyphae are abundantly formed. Inflated cells with refractive contents occur and measure (9.0–15.0)×(3.0–6.0) µm. Aerobic growth is pale to dark brownish orange-yellow, dull, flat, and with the margin entire or somewhat eroded.

Formation of ballistoconidia: Ballistoconidia are allantoid, falcate or cylindrical, (8.0–20.0)×(1.5–2.5) µm (Fig. 434).

Fermentation: absent.

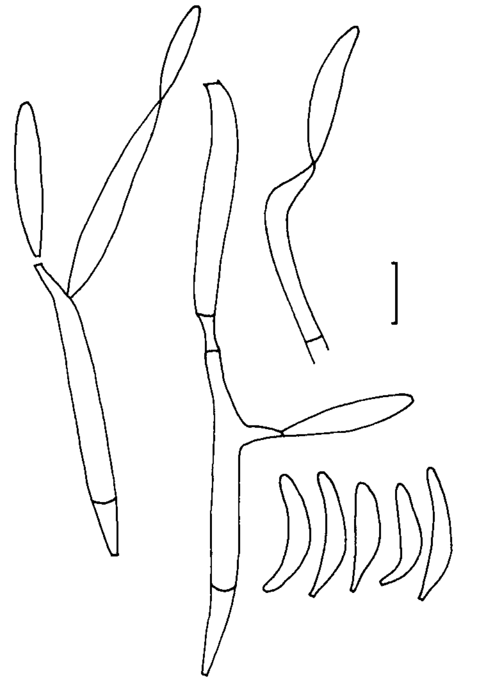


Fig. 434. *T. flava*, CBS 401.84. Hyphae and ballistoconidia on malt extract agar. Bar = 5 μm.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	–
L-Sorbose	+	Ethanol	–
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	+	D-Gluconate	–
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% (w/w) Glucose	–	Growth at 30°C	+
Starch formation	–	Growth at 35°C	–
Urease	+		

Co-Q: 10, CBS 401.84 (Boekhout et al. 1992a).
Mol% G + C: 63.2, one strain (T_m: Boekhout et al. 1992b).

Origin of the strain studied: CBS 401.84 (IFO 6833, UBC 917, YK 808), leaves of maple (*Acer* sp.), K. Tubaki, Japan.

Type strain: IFO 6833.

Comments: *Tilletiopsis flava* can be differentiated from both *T. fulvescens* and *T. minor* by its inability to grow on galactose, lactose, melezitose and erythritol (Boekhout 1991a). *Tilletiopsis flava* further differs from these species by electrophoretic enzyme patterns (Yamazaki et al. 1985), electrophoretic karyotypes and a mol% G + C of ca. 63 (Boekhout et al. 1992a,b).

109.3. *Tilletiopsis fulvescens* Gokhale (1972)

Growth on 5% malt extract agar: After 5 days at 17°C, hyphae are regularly branched, narrow, cylindrical, with cells measuring (15–60) × (1.0–2.5) μm, and with retraction septa (Fig. 435). Colonies are soft to rather dry, flat, warty or reticulate, glabrous or pruinose, dull, yellow-brown, and with an entire or eroded margin. A brown pigment is exuded into the agar.

Growth on the surface of assimilation media (glucose): Islets, a thin film and a sediment are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, abundant hyphae are formed. Chlamydospores are ellipsoidal, subglobose, cylindrical, and 4.0–8.0 μm in diameter. Aerobic growth is yellow or yellow-brown, dull, flat, and with the margin entire or eroded.

Formation of ballistoconidia: Ballistoconidia are allantoid or falcate, (5.0–16.0) × (1.0–2.5) μm (Fig. 435).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	–
Inulin	+	D-Gluconate	v
Soluble starch	+	DL-Lactate	v
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Nitrite	+
50% (w/w) Glucose	–	Growth at 30°C	+
Starch formation	–	Growth at 35°C	–
Urease	+		

Co-Q: 10, CBS 321.71 (Boekhout et al. 1992b).
Mol% G + C: 66.3–68.0, 4 strains (T_m: Boekhout et al. 1992a).

Origin of the strains studied: CBS 321.71 (ATCC 36405), *Oxycoccus microcarpus*, Madison, USA; CBS 607.83 (ATCC 24344, UBC 8006, YK 809) leaf of *Forsythia*, S. Reid, British Colombia, Canada.

Type strain: UBC 8006.

Comments: *Tilletiopsis fulvescens* is similar to *T. minor* and can be differentiated from the latter species by

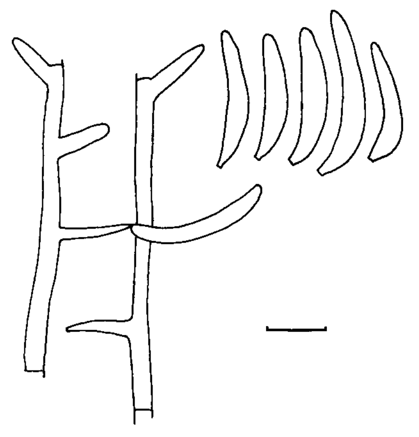


Fig. 435. *T. fulvescens,* CBS 607.83. Hyphae and ballistoconidia on malt extract agar. Bar = 5 μm.

a higher mol% G+C of ca. 66–68 (Boekhout 1991a, Boekhout et al. 1992b), and softer colonies. Moreover, the electrophoretic enzyme patterns of these species are dissimilar (Yamazaki et al. 1985).

109.4. *Tilletiopsis minor* Nyland (1950)

Growth at 5% malt extract agar: After 5 days at 17°C, hyphae are regularly branched, narrow, cylindrical, with cells of (10–75)×(1.0–2.0) μm, and with retraction septa. Chlamydospores occurring terminally or intercalarily, and are ellipsoidal, subglobose, fusiform, or cylindrical, (5.0–20.0)×(3.0–7.0) μm (Fig. 436). Colonies are tough, glabrous, pruinose or covered with slender fascicles, shiny or dull, cream to yellow-brown, and with an entire or eroded margin. A red brown pigment is exuded into the agar.

Growth on the surface of assimilation media: Islets, a ring and a small sediment are formed.

Dalmau plate on morphology agar: After 7 days at 17°C, hyphae are abundantly formed. Chlamydospores are similar to those on malt extract agar. Aerobic growth is yellowish-brown, dull or somewhat shiny, flat, convex or pulvinate, somewhat irregular, and with an entire or somewhat eroded margin.

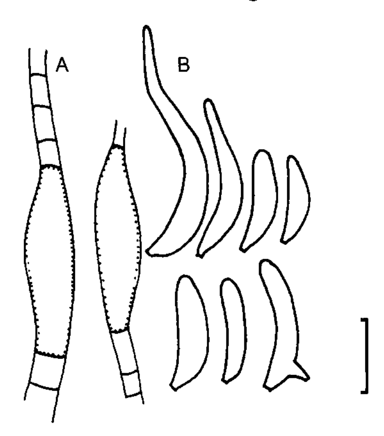


Fig. 436. *T. minor,* CBS 543.50. (A) Chlamydospores on malt extract agar. (B) Ballistoconidia on morphology agar. Bar = 5 μm.

Formation of ballistoconidia: Ballistoconidia are allantoid or falcate, (6.0–16.0)×(1.0–2.5) μm (Fig. 436).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	v
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	–
Inulin	+	D-Gluconate	v
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Nitrite	v
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	v
Urease	+	Growth at 35°C	–

Co-Q: 10, CBS 543.50, CBS 346.33 (Boekhout et al. 1992b).
Mol% G+C: 55.2–56.9, 12 strains (T_m: Boekhout et al. 1992a).

Origin of the strains studied: CBS 346.33 (ATCC 36406), origin unknown, H.G. Derx; CBS 543.50 (ATCC 10764, IMI 56590, YK 805), from leaves, G. Nyland, Washington, USA; CBS 440.61, leaves of *Taraxacum officinale,* Rothamsted, UK; CBS 264.82, powdery mildew, T. Hijwegen, Netherlands; CBS 604.83 (IFO 6904, UBC 915, YK 807), leaves, K. Tubaki, Tokyo, Japan: UAMH 2678, origin unknown, D. Westlake.

Type strain: CBS 543.50.

Comments: *Tilletiopsis minor* is an inhabitant of the phyllosphere. It has also been isolated from such diverse sources as air, industrial plants and medical specimen (see Boekhout 1991a). Reduction of growth of mildew by *T. minor* was observed under certain environmental conditions (Hijwegen 1986).

Tilletiopsis minor is characterized by dull, brownish, and somewhat pruinose colonies, which show limited expansion growth. *Tilletiopsis fulvescens,* another species with these characteristics, differs by a higher mol% G+C of ca. 66–68.

CBS 604.83 differs from other strains of *T. minor* by assimilation of erythritol (Boekhout 1991a), electrophoretic enzyme patterns (Yamazaki et al. 1985) and somewhat different karyotypes (Boekhout et al. 1992a).

109.5. *Tilletiopsis pallescens* Gokhale (1972)

Growth on 5% malt extract agar: After 5 days at 17°C, hyphae are regularly branched, narrow, cylindrical,

with cells of (20–80)×(1.5–3.0) µm, and with retraction septa. Chlamydospores occur terminally or intercalarily and are catenate or single, subglobose, ellipsoidal or cylindrical, (10–50)×(3.0–15.0) µm. Colonies are tough, flat, somewhat ridged or pustulate in the center, covered with slender fascicles, pale yellowish-brown or cream, and with an eroded margin.

Growth on the surface of assimilation media (glucose): A ring, islets and a film are formed.

Dalmau plate culture on morphology agar: After 7 days at 17°C, hyphae are abundantly formed. Chlamydospores are similar to those formed on malt extract agar. Aerobic growth is whitish, dull, flat, frequently with the center somewhat wrinkled or pustulate, pruinose, and with slender tapering fascicles, and an eroded margin.

Formation of ballistoconidia: Ballistoconidia are allantoid, falcate to cylindrical, (6.5–30.0)×(1.5–4.0) µm (Fig. 437).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	+	Ethanol	v
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	v
Inulin	v	D-Gluconate	v
Soluble starch	+	DL-Lactate	v
D-Xylose	v	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	v	Inositol	v
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	+

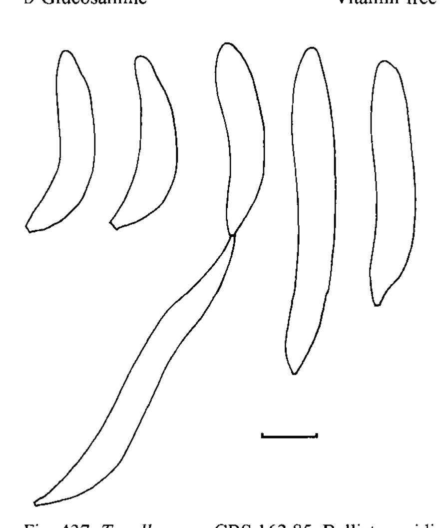


Fig. 437. *T. pallescens*, CBS 162.85. Ballistoconidia on malt extract agar. Bar = 5 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	v	Nitrite	v
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	v
Urease	+	Growth at 35°C	–

Co-Q: 10, CBS 606.83, CBS 369.85 (Boekhout et al. 1992b).
Mol% G+C: 42–45.5, 16 strains (T_m: Boekhout et al. 1992a).

Origin of the strains studied: CBS 606.83 (ATCC 24345, UBC 8007, YK 810), fruit-body of *Sirobasidium*, Shamoda, Japan; CBS 162.85, gall of *Clitocybe odorata*, H.A. van der Aa, Netherlands; CBS 364.85, CBS 367.85, CBS 368.85, all from powdery mildew cultures, J.P. Skou, Denmark; CBS 365.85 (ATCC 36535), powdery mildew, H.C. Hoch and R. Provvidenti, USA; CBS 438.90 (ATCC 66528), powdery mildew, A.L. Klecan, S. Hippe and S.C. Somerville, Michigan, USA.
Type strain: UBC 8007.

Comments: *Tilletiopsis pallescens* is similar in many aspects to *T. albescens,* but it can be differentiated from this species by colony appearance and a lower mol% G+C of ca. 42–45.5. *Tilletiopsis pallescens* is frequently isolated from other fungi, and has been reported to antagonize powdery mildews (Hoch and Provvidenti 1979, Hijwegen 1988, Klecan et al. 1990). Karyotypes of European strains, isolated from powdery mildew, were found to be similar, whereas the remaining strains showed considerable variation (Boekhout et al. 1992a).

109.6. *Tilletiopsis washingtonensis* Nyland (1950)

Synonyms:
Tilletiopsis cremea Tubaki (1952)
Tilletiopsis lilacina Tubaki (1952)

Growth on 5% malt extract agar: After 5 days at 17°C, hyphae are regularly branched, narrow, with cells of (20–100)×(1.0–3.0) µm, and with retraction septa (Fig. 438). Chlamydospores occur terminally or intercalarily and are subglobose, ellipsoid or cylindrical, and frequently catenate, (6.0–35.0)×(2.5–10.0) µm. Colonies are soft, butyrous flat, usually glabrous, shiny or dull, yellowish-cream or pale pinkish yellow-brown, and with the margin eroded.

Growth on the surface of assimilation media (glucose): A ring, film and a sediment are formed.

Dalmau plate on morphology agar: After 7 days at 17°C, hyphae are abundantly formed. Chlamydospores are similar to those formed on malt extract agar. Aerobic growth is pale pinkish-cream, dull or shiny flat, pustulate, ridged or reticulate, and with the margin entire, straight or lobate.

Formation of ballistoconidia: Ballistoconidia are formed singly or in small groups, and are allantoid, falcate or cylindrical, (8.0–24.0)×(1.0–3.5) µm (Fig. 438).

Fermentation: absent.

Assimilation (17°C):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	–
L-Sorbose	–	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	v	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	v	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	v
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	–	Nitrite	+
50% (w/w) Glucose	–	Growth at 25°C	+
Starch formation	–	Growth at 30°C	v
Urease	+	Growth at 35°C	–

Co-Q: 10, CBS 544.50, CBS 605.83 (Boekhout et al. 1992b).
Mol% G+C: 66.5–70.0, 18 strains (T_m: Boekhout et al. 1992a).

Origin of the strains studied: CBS 544.50 (ATCC 36489, YK 803), leaves of *Rubus idaeus,* G. Nyland, Washington, USA; CBS 603.83 (IFO 6832, UBC 925, YK 811), leaves, K. Tubaki, Japan; CBS 605.83 (IFO 6831, NI 3113, UBC 912, YK 809), leaves, K. Tubaki, Japan; UAMH 1738, authentic strain from G. Nyland, Washington, USA.

Type strain: CBS 544.50.

Comments: *Tilletiopsis washingtonensis* seems to be a common inhabitant of the phyllosphere (Brady 1960, Pady 1974, Tubaki 1952). Nyland (1950) considered *T. washingtonensis* to be identical with Derx's (1948) *Tilletiopsis* species 4. *Tilletiopsis washingtonensis, T. cremea* and *T. lilacina* are considered conspecific because of morphological, physiological, and biochemical similarities (Boekhout 1991a). The consistency of the colonies, considered an important taxonomic criterion by Tubaki (1952), seems of insignificant value. Considerable heterogeneity was observed with respect to electrophoretic enzyme (Yamazaki et al. 1985) and karyotype patterns (Boekhout et al. 1992a).

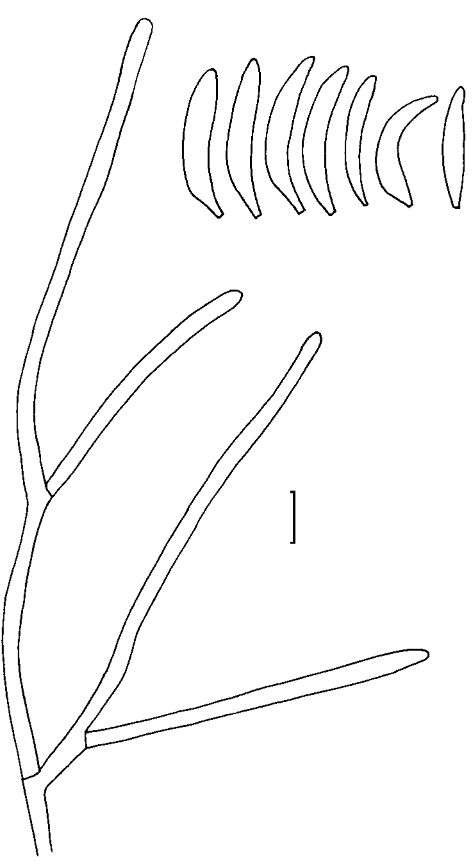


Fig. 438. *T. washingtonensis,* CBS 544.50. Hyphae and ballistoconidia on morphology agar. Bar = 5 μm.

Comments on the genus

The name *Tilletiopsis* was published by Derx (1930). This author failed to publish a generic definition or to correctly describe the species (Derx 1930, 1948). Nyland circumscribed the genus by designating *T. washingtonensis* Nyland as neotype.

Anamorphs of Tilletiales, e.g., *Entyloma, Melanotaenium* and *Tilletia,* and probably species belonging to other genera as well, are morphologically similar to *Tilletiopsis* (Boekhout 1991a). Therefore, *Tilletiopsis* was regarded to be phylogenetically related to the Tilletiales (Boekhout et al. 1992b).

110. *Trichosporon* Behrend

E. Guého, M.Th. Smith and G.S. de Hoog

Diagnosis of the genus

Colonies are cream-colored, moist or dry, and with or without a white, farinose covering. True mycelium is present; pseudomycelium is absent. Arthroconidia are usually abundant; lateral, clavate conidia also may be present. Appressoria, sarcinae, fusiform giant cells and endoconidia are occasionally present. Hyphal septa are mostly with dolipores which may be with or without vesicular or tubular parenthesomes. Sexual reproduction is absent.

Fermentation is negative. The Diazonium blue B reaction is positive. Urea is hydrolyzed. Coenzyme Q-9 or Q-10 are formed. Xylose is present in whole-cell hydrolyzates.

Type species

Trichosporon ovoides Behrend

Species accepted

1. *Trichosporon aquatile* Hedrick & Dupont (1968)
2. *Trichosporon asahii* Akagi ex Sugita, Nishikawa & Shinoda (1994)
3. *Trichosporon asteroides* (Rischin) Ota (1926)
4. *Trichosporon brassicae* Nakase (1971)
5. *Trichosporon coremiiforme* (M. Moore) Guého & M.Th. Smith (1992)
6. *Trichosporon cutaneum* (de Beurmann, Gougerot & Vaucher) Ota (1926)
7. *Trichosporon dulcitum* (Berkhout) Weijman (1979)
8. *Trichosporon faecale* (Batista & Silveira) Guého & M.Th. Smith (1992)
9. *Trichosporon gracile* (Weigmann & Wolff) Guého & M.Th. Smith (1992)
10. *Trichosporon inkin* (Oho ex Ota) do Carmo-Sousa & van Uden (1967)
11. *Trichosporon jirovecii* Frágner (1969)
12. *Trichosporon laibachii* (Windisch) Guého & M.Th. Smith (1992)
13. *Trichosporon loubieri* (Morenz) Weijman (1979)
14. *Trichosporon moniliiforme* (Weigmann & Wolff) Guého & M.Th. Smith (1992)
15. *Trichosporon montevideense* (Aciole de Queiroz) Guého & M.Th. Smith (1992)
16. *Trichosporon mucoides* Guého & M.Th. Smith (1992)
17. *Trichosporon ovoides* Behrend (1890)
18. *Trichosporon pullulans* (Lindner) Diddens & Lodder (1942)
19. *Trichosporon sporotrichoides* (van Oorschot) van Oorschot & de Hoog (1981)

Key to species

See Table 95.

1.	a	Growth with nitrate	*T. pullulans*:	p. 869
	b	Absence of growth with nitrate → 2		
2(1).	a	Growth with creatinine → 3		
	b	Absence of growth with creatinine → 4		
3(2).	a	Growth at 30°C	*T. laibachii*:	p. 865
	b	Absence of growth at 30°C	*T. sporotrichoides*:	p. 870
4(2).	a	Growth at 30°C → 5		
	b	Absence of growth at 30°C	*T. dulcitum*:	p. 861
5(4).	a	Growth with erythritol → 6		
	b	Absence of growth with erythritol → 17		
6(5).	a	Growth with melibiose → 7		
	b	Absence of growth with melibiose → 10		
7(6).	a	Growth with galactitol → 8		
	b	Absence of growth with galactitol → 9		
8(7).	a	Growth with glucono-δ-lactone	*T. mucoides*:	p. 868
	b	Absence of growth with glucono-δ-lactone	*T. jirovecii*:	p. 864
9(7).	a	Growth with glucono-δ-lactone; species mostly isolated from soil and water	*T. moniliiforme*:	p. 866
	b	Absence of growth with glucono-δ-lactone; species mostly isolated from humans	*T. cutaneum*:	p. 860
10(6).	a	Growth with L-arabinitol → 11		
	b	Absence of growth with L-arabinitol → 15		

Table 95
Key characters of species in the genus *Trichosporon*

Species	Assimilation[a]														Growth				Source[b]	
	Gal	Rha	Mel	Mlz	Gly	Ery	Ara	Glt	Ino	GδL	Nit	Cre	Gln	Sor	30°C	35°C	37°C	42°C	Human	Soil
Trichosporon aquatile	+	−	−	v	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	+
T. asahii	+	+	−	v	v	+	+	−	v	v	−	−	+	v	+	+	+	−	+	−
T. asteroides	+	+	−	+	+	+	+	−	+	−	−	−	v	v	+	n	v	−	+	−
T. brassicae	+	−	−	−	−	−	−	−	+	+	−	−	+	+	+	−	−	−	−	−
T. coremiiforme	+	+	−	+	v	+	+	v	+	+	−	−	−	v	+	+	+	−	+	−
T. cutaneum	+	+	+	+	+	+	+	−	+	−	−	−	v	v	+	−	−	−	+	−
T. dulcitum	−	+	−	+	+	−	v	v	+	−	−	−	v	v	−	−	−	−	−	+
T. faecale	+	+	−	+	+	+	−	v	+	+	−	−	+	−	+	+	+	−	+	−
T. gracile	−	−	−	−	+	−	−	−	+	v	−	−	v	v	+	v	−	−	−	−
T. inkin	v	−	−	+	v	+	−	−	+	v	−	−	v	v	+	+	+	v	+	−
T. jirovecii	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	v	−	−	−	−
T. laibachii	+	+	+	v	+	−	v	+	+	v	−	+	+	+	+	−	−	−	−	+
T. loubieri	+	+	+	−	+	−	+	−	+	+	−	−	+	v	+	+	+	+	−	−
T. moniliiforme	+	+	+	+	+	+	+	−	+	v	−	−	v	v	+	v	−	−	−	+
T. montevideense	+	−	−	+	+	−	v	+	+	+	−	−	v	−	+	+	−	−	+	+
T. mucoides	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	−
T. ovoides	+	+	−	v	v	+	−	−	+	v	−	−	v	v	+	+	v	−	+	−
T. pullulans	+	v	+	v	v	+	−	−	+	+	+	−	−	v	−	−	−	−	−	−
T. sporotrichoides	+	+	+	+	+	−	−	−	+	+	−	+	+	+	−	−	−	−	−	+

[a] Abbreviations: Gal, galactose; Rha, L-rhamnose; Mel, melibiose; Mlz, melezitose; Gly, glycerol; Ery, erythritol; Ara, L-arabinitol; Glt, galactitol; Ino, inositol; GδL, glucono-δ-lactone; Nit, nitrate; Cre, creatinine; Gln, D-glucosamine, Sor, L-sorbose.
[b] Human, primary source is human; soil, primary source is soil and water.

11(10). a Growth with glucono-δ-lactone → 12
b Absence of growth with glucono-δ-lactone → 14
12(11). a Growth with D-glucosamine → 13
b Absence of growth with D-glucosamine .... *T. coremiiforme*: p. 859
13(12). a Growth with L-sorbose .... *T. asahii*: p. 857
b Absence of growth with L-sorbose .... *T. faecale*/*T. asahii*: p. 862
14(11). a Growth with D-glucosamine .... *T. asahii*/*T. asteroides*: p. 857
b Absence of growth with D-glucosamine .... *T. asteroides*: p. 858
15(10). a Growth with inositol and at 35°C → 16
b Absence of growth with inositol and at 35°C .... *T. aquatile*: p. 856
16(15). a Growth with L-rhamnose .... *T. ovoides*: p. 869
b Absence of growth with L-rhamnose .... *T. inkin*: p. 863
17(5). a Growth at 42°C .... *T. loubieri*: p. 866
b Absence of growth at 42°C → 18
18(17). a Growth at 37°C .... *T. asahii*: p. 857
b Absence of growth at 37°C → 19
19(18). a Growth with melezitose → 20
b Absence of growth with melezitose → 21
20(19). a Growth with melibiose, no growth at 35°C .... *T. multisporum*, see under *T. laibachii*: p. 865
b Absence of growth with melibiose, growth at 35°C .... *T. montevideense*: p. 867
21(19). a Growth with D-galactose, absence of growth with glycerol .... *T. brassicae*: p. 859
b Absence of growth with D-galactose, growth with glycerol .... *T. gracile*: p. 862

Systematic discussion of the species

110.1. *Trichosporon aquatile* Hedrick & Dupont (1968)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are pale cream-colored, mat, smoothly cerebriform or volcano-shaped, odorless, and 6–8 mm in diameter. Hyphae are flexuose, frequently branched at wide angles and finally disarticulating into narrow arthroconidia of variable length; terminal conidia are often clavate, 3×(6–14) μm (Fig. 439).

Fermentation: absent.

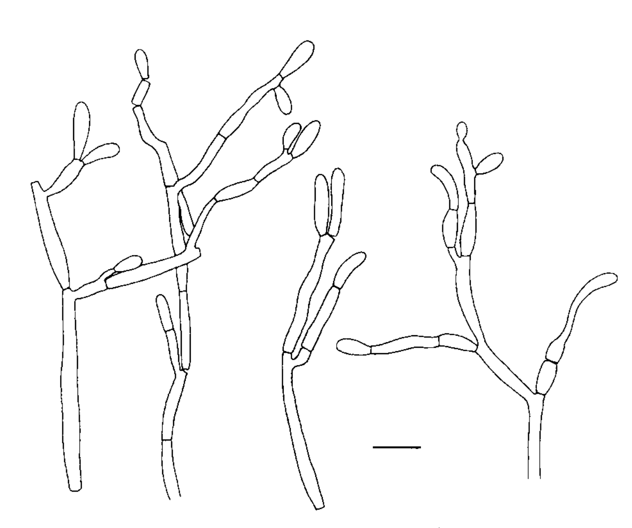


Fig. 439. *T. aquatile*, CBS 5988. Flexuous hyphal cells with clavate conidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	–
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	v	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	–	Nitrite	–
L-Arabinitol	–	0.01% Cycloheximide	+
Glucono-δ-lactone	–	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–
Ethylamine	+		

Co-Q: 9, CBS 5973 and 5988 (Hara et al. 1989, Guého et al. 1992b).
Mol% G+C: 62.9–62.4, CBS 5973 and 5988 (T_m: Guého et al. 1992b); 64.2, CBS 5973 (BD: Guého et al. 1984).

Origin of the strains studied: CBS 5973, water, ex NRRL; CBS 5988, water, Hedrick.
Type strain: CBS 5973.

Comments: The species is morphologically characterized by flexuose hyphae which are often branched at right angles. With the exception of erythritol, most higher

alcohols are not assimilated. The species shows no growth with L-sorbose and inositol.

110.2. *Trichosporon asahii* Akagi ex Sugita, Nishikawa & Shinoda (1994)

Synonyms:

Trichosporon asahii Akagi (1929) nom. inval.
?*Proteomyces infestans* Moses & Vianna (1913)
?*Sporotrichum infestans* (Moses & Vianna) Sartory (1923)
?*Mycoderma infestans* (Moses & Vianna) da Fonseca & de Arêa Leao (1929)
?*Trichosporon infestans* (Moses & Vianna) Ciferri & Redaelli (1935)
?*Geotrichum infestans* (Moses & Vianna) Brumpt (1936)
?*Trichosporon cutaneum* (de Beurmann, Gougerot & Vaucher) Ota var. *infestans* (Moses & Vianna) Diddens & Lodder (1942)
Trichosporon cutaneum (de Beurmann, Gougerot & Vaucher) Ota var. *peneaus* Phaff, Mrak & Williams (1952)
Trichosporon figueirae Batista & Silveira (1960)
Trichosporon loboi Batista, Campos & Oliveira (1963)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are 16–24 mm in diameter, white, farinose at the center, with a wide, dry, often finely zonate margin with deep transverse fissures. Odor is lacking or faintly cheese-like. Arthroconidia are abundant, rectangular, mostly 2–3 μm wide, and with rounded ends, (3–4)×(4–7) μm (Fig. 440). Lateral conidia are absent. Appressoria are absent.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	v	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	v
Trehalose	v	Galactitol	–
Lactose	+	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	v
D-Xylose	v	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	+	Inositol	v
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	v	Nitrite	v
L-Arabinitol	+	0.01% Cycloheximide	+
Glucono-δ-lactone	v	0.1% Cycloheximide	v
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 37°C	+
Ethylamine	+	Growth at 42°C	–

Co-Q: 9, CBS 2479, CBS 2530, CBS 4829 and 6 additional strains (Hara et al. 1989, Guého et al. 1992b, Sugita et al. 1994).

Mol% G+C: 57.8–58.7, CBS 2479, CBS 2497, CBS 2530, CBS 4829, CBS 5286 and 20 additional strains (T_m: Guého et al. 1992b); 59.9, CBS 2530 (BD: Guého et al. 1984); 59.8, CBS 2479, CBS 2939, CBS 4829 (HPLC: Sugita et al. 1994).

Origin of the strains studied: CBS 2479, nail of patient with psoriasis, Ota, type strain of *T. asahii*; CBS 2497, shrimp, Phaff, type strain of *T. cutaneum* var. *peneaus*; CBS 2530, mouse inoculated with pus, Ciferri, type strain of *T. infestans*; CBS 4829, human feces, Batista, type strain of *T. figueirae*; CBS 5286, skin lesion, Batista, type strain of *T. loboi*; blood (6), urine (2), lymphoma (2), mare uterus (1), animal white piedra (2), inflamed gland (1), tofu cake (1); TIMM 1318, SHP patient's environment, Ando.

Type strain: CBS 2479.

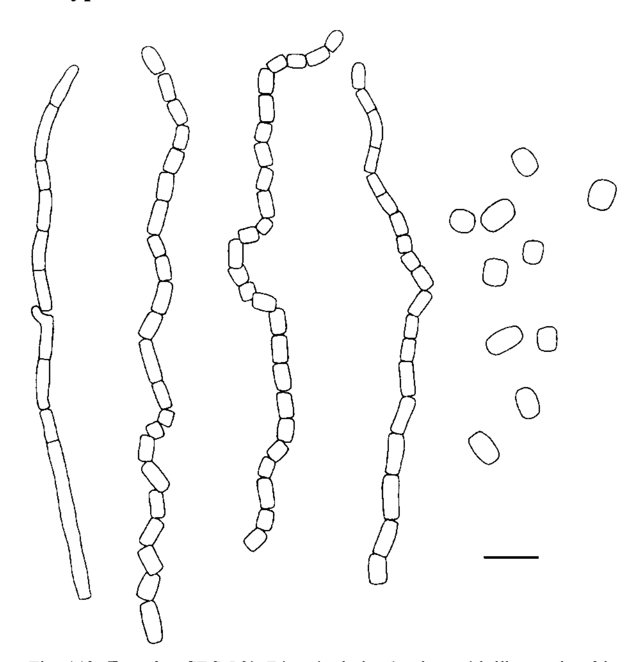


Fig. 440. *T. asahii*, CBS 561. Disarticulating hyphae with liberated, cubic arthroconidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Comments: Fresh isolates may be creamy, attaining their characteristic dry colony appearance only after repeated transfers. The species grows with D-glucosamine and L-arabinitol but not with raffinose. Physiologically it cannot be distinguished from *T. asteroides*. *Trichosporon asahii* is close to *T. ovoides* and *T. inkin*, but differs by the absence of appressoria and by its ecological preferences. Physiologically it can be recognized from its growth with L-rhamnose and L-arabinitol.

The type strain of *Trichosporon infestans*, CBS 2530, differs by its absence of growth with D-xylose, trehalose and erythritol. However, it proved to be identical to *T. asahii* on the basis of DNA homology and partial rRNA sequences (Guého et al. 1992b) and is therefore listed as a doubtful synonym. Hara et al. (1989) reported Co-Q 10 for CBS 2530, while Guého et al. (1992b) found Co-Q 9.

Trichosporon asahii is mostly found as a causative agent of disseminated mycoses in patients with impaired

natural immunity. Occasionally it causes human or animal white piedra. Only a very few strains are known from non-vertebrate sources.

The species in the above sense comprises agents of human mycoses which in the literature are mostly referred to as *Trichosporon beigelii*. This name was abandoned because the identity of the type specimen could not be established (Guého et al. 1992a). Causative agents of capital white piedra are now known as *Trichosporon ovoides*. Guého et al. (1992a) stressed that *T. cutaneum*, which has widely been regarded as a synonym of *T. beigelii*, is in fact another species, having coenzyme Q-10 rather than Q-9. *T. cutaneum* can be distinguished from *T. asahii* by the absence of growth at 37°C, but it grows with melibiose and raffinose. Strain TIMM 1318 was designated as the standard for serotype II recognized within the genus (Nishiura et al. 1996).

110.3. *Trichosporon asteroides* (Rischin) Ota (1926)

Synonyms:

Parendomyces asteroides Rischin (1921)
Geotrichoides asteroides (Rischin) Langeron & Talice (1932)
Proteomyces asteroides (Rischin) Dodge (1935)
[nec *Monilia asteroides* (Castellani) Castellani & Chalmers (1919) = *Mycoderma asteroides* (Castellani) Brumpt (1927) = *Geotrichum asteroides* (Castellani) Basgal (1931)]

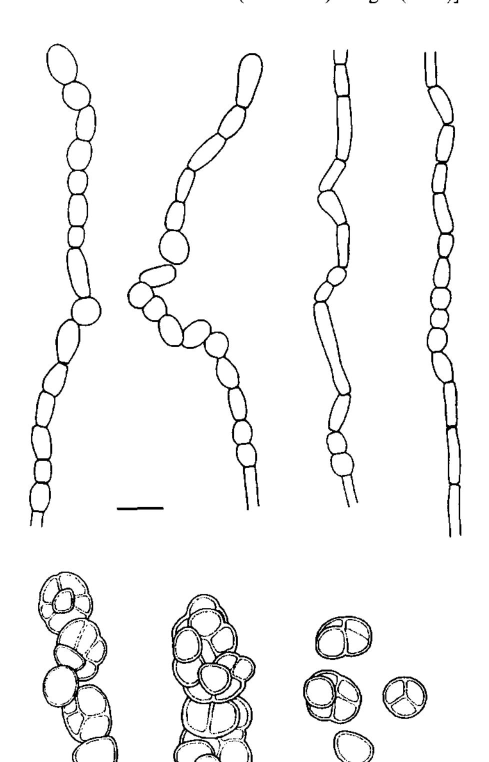


Fig. 441. (Top) *T. asteroides*, CBS 2481. Hyphae disarticulating into arthroconidia. (Bottom) *Fissuricella filamenta*, CBS 7624. Meristematic conidiation. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

?*Prototheca filamenta* Arnold & Ahearn (1972)
?*Fissuricella filamenta* (Arnold & Ahearn) Pore, d'Amato & Ajello (1977)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are undulate, mat, whitish, with a radially furrowed margin and 10 mm in diameter. True hyphae are present and break into one-celled or septate hyphal elements (3–4) × (4–15) μm (Fig. 441).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	v
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	v	Urease	+
Xylitol	+	Nitrite	–
L-Arabinitol	+	0.01% Cycloheximide	v
Glucono-δ-lactone	–	0.1% Cycloheximide	v
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 37°C	v
Ethylamine	+	Growth at 42°C	–

Co-Q: 9, CBS 2481, CBS 6183 and CBS 7623 (Hara et al. 1989, Guého et al. 1992b).

Mol% G + C: 61.5–62.0, CBS 2481, CBS 6183 and 4 additional strains (T_m: Guého et al. 1992b).

Origin of the strains studied: CBS 2481, human skin, Beintema; CBS 6183, bovine mastitis, van der Walt; CBS 7623 (ATCC 22432), skin, Ahearn, type strain of *P. filamenta*; blood (1); skin (2); unknown source (1).

Type strain: CBS 2481.

Comments: The species can be recognized physiologically by growth with D-galactose, L-rhamnose, erythritol and L-arabinitol but not with glucono-δ-lactone. It cannot be distinguished physiologically from *T. asahii*, but in contrast, cultures are filamentous with the mycelium locally disarticulating into continuous or septate hyphal elements of variable size.

Two strains of *Fissuricella filamenta*, isolated from human skin, showed DNA homology values around 85% with *T. asteroides* strains (Guého et al. 1992b). The groups of strains are consequently genetically close, despite the

fact that the *F. filamenta* strains were entirely composed of meristematic cells, while such cells were absent from the strains of *T. asteroides*. Colonies of *F. filamenta* are dry, finely cerebriform without a differentiated marginal zone, 5–6 mm diameter, finally become brownish, and crack the agar medium. Possibly *F. filamenta* is a meristematic mutant of *T. asteroides*. The meristematic growth of *F. filamenta* was reminiscent of the sarcinae known in *Trichosporon inkin in vivo* or obtained after cultivation on media rich in sugar. For this reason, synonymy of the two species was proposed by Pore et al. (1977). However, *T. inkin* forms appressoria and is unable to grow with L-rhamnose, xylitol and L-arabinitol.

110.4. *Trichosporon brassicae* Nakase (1971a)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are whitish, shiny, slightly moist, flat, pustular to cerebriform, and 19 mm in diameter. Hyphae are present and easily fall apart into elements of variable length which are rectangular, (4–8)×(10–30) μm (Fig. 442).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	–
Maltose	+	Erythritol	–
Cellobiose	–	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	–	Urease	+
Xylitol	–	Nitrite	–
L-Arabinitol	–	0.01% Cycloheximide	+
Glucono-δ-lactone	+	0.1% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	+
Ethylamine	+	Growth at 37°C	–

Co-Q: 9, CBS 6382 (Hara et al. 1989, Guého et al. 1992b).
Mol% G+C: 54.9, CBS 6382 (T_m: Nakase 1971a, T_m: Guého et al. 1992b); 56.9 (BD: Guého et al. 1984).

Origin of the strain studied: CBS 6382, cabbage, Nakase.

Type strain: CBS 6382.

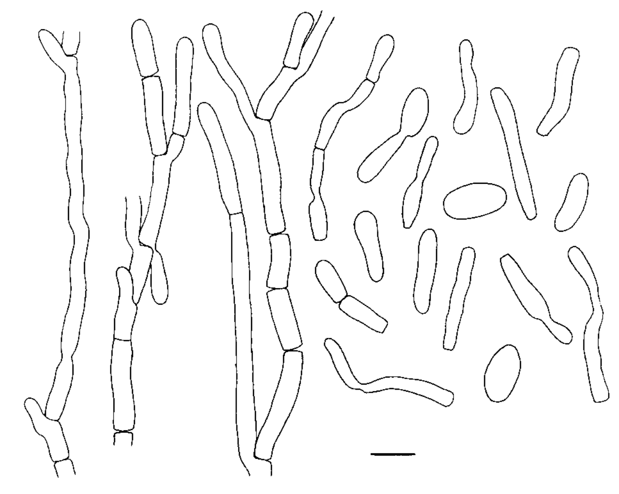


Fig. 442. *T. brassicae*, CBS 6382. Disarticulating hyphae and liberated, partly inflated, hyphal elements. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Comments: *T. brassicae* differs from all other *Trichosporon* species by its inability to assimilate cellobiose. With TEM, no septal pores were found, but septa were often swollen and of spongy texture (Guého et al. 1992b).

110.5. *Trichosporon coremiiforme* (M. Moore) Guého & M.Th. Smith (Guého et al. 1992b)

Synonym:
Hemispora coremiiformis M. Moore (1935)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are cream-colored, dry, mat, nearly flat, odorless, and 11 mm in diameter, with a finely fimbriate margin. Hyphae are often present in fine tufts and branchlets; hyphae mainly disarticulate into arthroconidia, 3×(6–10) μm (Fig. 443).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	–	Vitamin-free	–

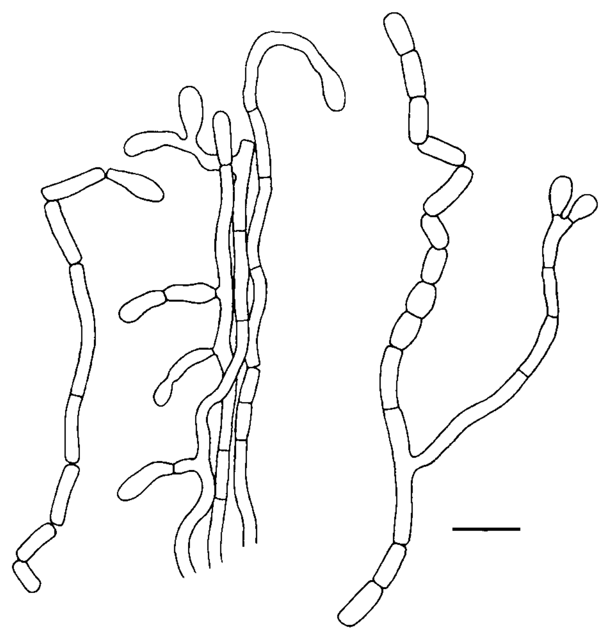


Fig. 443. *T. coremiiforme*, CBS 2482. Disarticulating hyphae, partly in small bundles. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	L-Lysine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	+
L-Arabinitol	+	0.01% Cycloheximide	+
Glucono-δ-lactone	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 37°C	+
Ethylamine	+	Growth at 42°C	–

Co-Q: 9, CBS 2482 (Hara et al. 1989, Guého et al. 1992b, Sugita et al. 1994).

Mol% G + C: 59.3, CBS 2482 (T_m: Guého et al. 1992b); 60.3, CBS 2482 (HPLC: Sugita et al. 1994).

Origin of the strains studied: CBS 2482, head lesion, Moore, type strain of *H. coremiiformis*.

Type strain: CBS 2482.

Comments: *T. coremiiforme* has the following unique combination of physiological characters: growth with L-rhamnose, α-methyl-D-glucoside, L-arabinitol and glucono-δ-lactone; absence of growth with D-glucosamine and melibiose. Sugita et al. (1994) proposed *T. coremiiforme* as a variety of *T. asahii* on the basis of 56% DNA relatedness of respective type cultures (CBS 2482 and CBS 2479).

110.6. *Trichosporon cutaneum* (de Beurmann, Gougerot & Vaucher) Ota (1926)

Synonyms:

Oidium cutaneum de Beurmann, Gougerot & Vaucher (de Beurmann and Gougerot 1909)
Monilia cutanea (de Beurmann, Gougerot & Vaucher) Castellani & Chalmers (1913)
Mycoderma cutaneum (de Beurmann, Gougerot & Vaucher) Brumpt (1913)
Geotrichoides cutaneus (de Beurmann, Gougerot & Vaucher) Langeron & Talice (1932)
Geotrichum cutaneum (de Beurmann, Gougerot & Vaucher) de Almeida (1933)
Proteomyces cutaneus (de Beurmann, Gougerot & Vaucher) Dodge (1935)
Basidiotrichosporon cutaneum (de Beurmann, Gougerot & Vaucher) Kocková-Kratochvílová, Sláviková & Zemek (1977b) nom. nud.
Geotrichoides paludosus Smit (1934)
Trichosporon minor de Arêa Leão (1940) nom. nud.

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are cream-colored, cerebriform, with broad, moist, glistening marginal zones, and 15–17 mm in diameter; the odor is cheese-like. Cultures generally consist of disarticulating hyphae, but may revert to yeast growth with subspheroidal budding cells. Arthroconidia are regular, (3–4) × (4–8) µm, with lateral, clavate blastoconidia (Fig. 444).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	–
L-Arabinitol	+	0.01% Cycloheximide	v
Glucono-δ-lactone	–	0.1% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	–
L-Lysine	+		

Co-Q: 10, CBS 2466, CBS 2480 and CBS 2545 (Hara et al. 1989, Guého et al. 1992b); 10, CBS 2466 (Yamada et al. 1982).

Mol% G + C: 60.7–61.2, CBS 2466, CBS 2480, CBS 2545 (T_m: Guého et al. 1992b); 63.5, CBS 2466 (BD: Guého et al. 1984).

Origin of the strains studied: CBS 2466, skin lesion, Langeron; CBS 2480, axillary white piedra, de Arêa Leao, type strain of *T. minor*; CBS 2545, activated sludge, Smit, type strain of *G. paludosus*.

Type strain: CBS 2466.

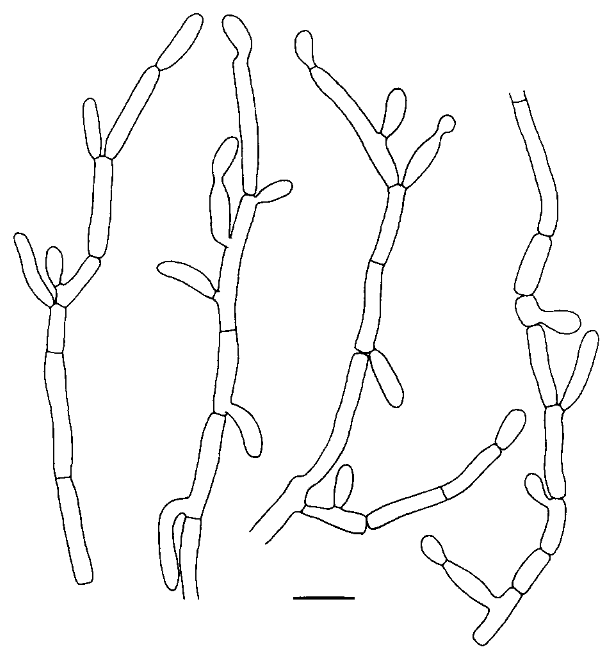


Fig. 444. *T. cutaneum,* CBS 2466. Disarticulating, branched hyphae. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

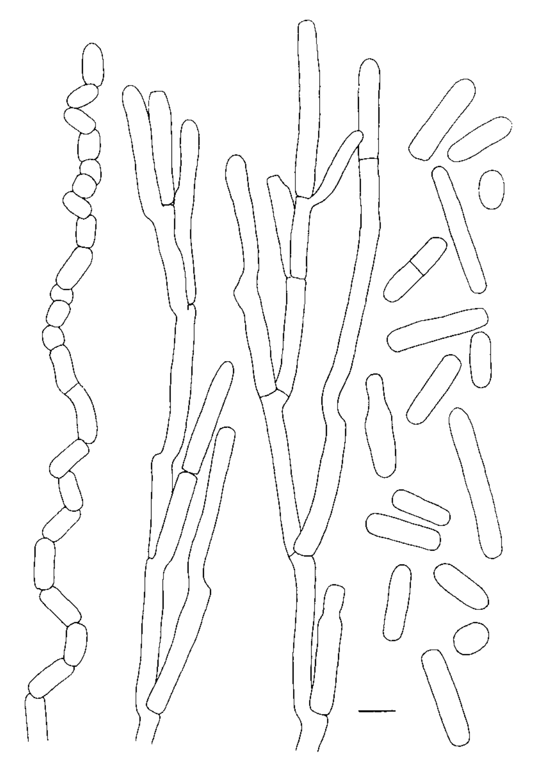


Fig. 445. *T. dulcitum,* CBS 7608. Hyphae disarticulating into arthroconidia; hyphae with cymose branching. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Comments: Key characters are the absence of growth with galactitol and glucono-δ-lactone; however, the species cannot be distinguished easily from *T. moniliiforme* by differences in growth reactions. *T. moniliiforme* shows variable growth reactions with glucono-δ-lactone. *Trichosporon cutaneum* is more sensitive to cycloheximide, not growing after two days of incubation with 0.01%, and to elevated temperature, not being able to grow at 35°C. The two species differ in their ecology; *T. moniliiforme* is restricted to natural sources such as soil. In contrast to the opinion held in the older literature, *T. cutaneum sensu stricto* appears to be a rare species (Guého et al. 1992b) and mostly occurs as an agent of superficial mycoses of warm-blooded animals.

110.7. *Trichosporon dulcitum* (Berkhout) Weijman (1979b)

Synonyms:
Oospora dulcita Berkhout (1923)
Geotrichum dulcitum (Berkhout) Windisch (1952)
Protendomycopsis domschii Windisch (1965)
Schizosaccharomyces zambettakesii Ramírez Gómez (1957)
Geotrichum zambettakesii Ramírez Gómez (1959)
Endomyces zambettakesii (Ramírez Gómez) Novák & Zsolt (1961)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are cream-colored, moist, flat, with shallow central depressions, and 25–28 mm in diameter. The growth has a strong smell of rotten cauliflower. Large hyphae with cymose branching are often present. Arthroconidia are formed and become subspheroidal to cylindroidal, (5–8)×(10–40) μm (Fig. 445).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	v
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	–
L-Arabinitol	–	0.01% Cycloheximide	+
Glucono-δ-lactone	+	0.1% Cycloheximide	–
Cadaverine	+	Growth at 25°C	+
Creatinine	–	Growth at 30°C	–
L-Lysine	+		

Co-Q: 9, CBS 8257, CBS 5785 and CBS 5786 (Hara et al. 1989, Guého et al. 1992b).
Mol% G+C: 54.9–55.4, CBS 8247, CBS 5786 and 2 additional strains (T_m: Guého et al. 1992b); 57.5, CBS 8257 (BD: Guého et al. 1984).

Origin of the strains studied: CBS 8257, soil, Den Dooren de Jong; CBS 5785, toadstool, Ramírez, type strain of *S. zambettakesii*; CBS 5786, soil, Windisch, type strain of *P. domschii*; CBS 7608, loess soil, Nirenberg.

Type strain: CBS 8257.

Comments: The species is an inhabitant of soil. It is mesophilic with optimum growth at 18°C. *Schizosaccharomyces zambettakesii* was found to be a synonym on the basis of DNA reassociation (Guého et al. 1992b), but in contrast to other strains of the species, it does not grow with citrate as sole carbon source.

110.8. *Trichosporon faecale* (Batista & Silveira) Guého & M.Th. Smith (Guého et al. 1992b)

Synonym:
Endomycopsis mali (Lewis) Dekker var. *faecalis* Batista & Silveira (1959b)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are cream-colored, moist, irregularly cerebriform at the center, flat or with radial fissures at the margin, and with some areas having a whitish, hispid covering. True hyphae are present, and disarticulate into separate cells measuring 2.5×(3.5–9.0) μm (Fig. 446).

Fermentation: absent.

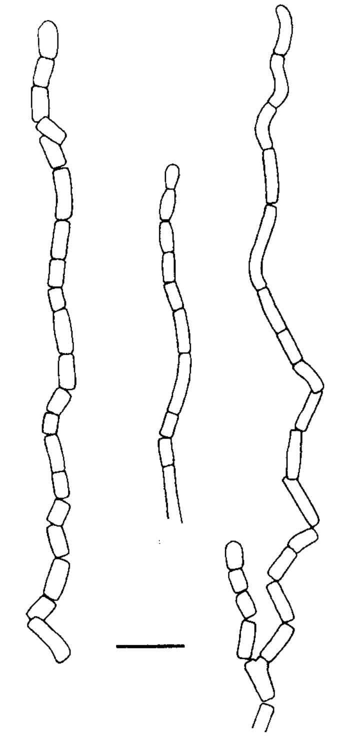


Fig. 446. *T. faecale*, CBS 4828. Hyphae disarticulating into arthroconidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	–
L-Arabinitol	+	0.01% Cycloheximide	+
Glucono-δ-lactone	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 37°C	+
L-Lysine	+	Growth at 42°C	–

Co-Q: 9, CBS 4828 (Hara et al. 1989, Guého et al. 1992b, Sugita et al. 1994).
Mol% G+C: 58.7, CBS 4828 (T_m: Guého et al. 1992b); 60.0, CBS 4828 (HPLC: Sugita et al. 1994).

Origin of the strain studied: CBS 4828, human feces, Batista, type strain of *E. mali* var. *faecalis*.

Type strain: CBS 4828.

Comments: The species is close to *T. ovoides*, *T. inkin* and *T. aquatile* on the basis of DNA reassociation data (Guého et al. 1992b), but can be distinguished by its unique combination of physiological characters: assimilation of D-glucosamine, D-arabinose, L-rhamnose, ribitol and glucono-δ-lactone but lack of growth with L-sorbose and melibiose. As for *T. coremiiforme*, Sugita et al. (1994) proposed *T. faecale* as a variety of *T. asahii* on the basis of 53% DNA relatedness of respective type cultures (CBS 4828 and CBS 2479).

110.9. *Trichosporon gracile* (Weigmann & Wolff) Guého & M.Th. Smith (Guého et al. 1992b)

Synonyms:
Oidium gracile Weigmann & Wolff (1909)
Oospora gracile (Weigmann & Wolff) Berkhout (1923)
Geotrichum gracile (Weigmann & Wolff) Windisch (1952)
Geotrichum vanrijiae Saëz (1964)
Trichosporon lutetiae Saëz (1977)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are glassy-whitish, mat and smooth, nearly flat, with slight, shallow depressions at

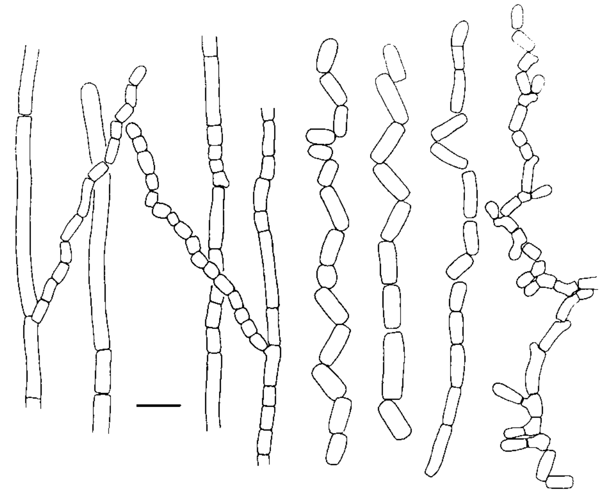


Fig. 447. *T. gracile*, CBS 8189. Hyphae disarticulating into arthroconidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 µm.

the center, and measure 29–30 mm in diameter. The odor is cheese-like. True hyphae are present and easily disarticulate into regularly rectangular arthroconidia that measure 3×(4–12) µm (Fig. 447).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	v	Glycerol	+
Maltose	v	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	v	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	+
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	v	Urease	+
Xylitol	v	Nitrite	–
L-Arabinitol	–	0.01% Cycloheximide	+
Glucono-δ-lactone	v	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	v
L-Lysine	+	Growth at 37°C	–

Co-Q: 9, CBS 8189, CBS 8193, CBS 6861 and CBS 7609 (Hara et al. 1989, Guého et al. 1992b); 9, CBS 6861, CBS 8193 (Sugita et al. 1994).

Mol% G+C: 57.6–58.0, CBS 6861, CBS 8189, CBS 8193 and 3 additional strains (T_m: Guého et al. 1992b); 59.7–60.0, CBS 6861, CBS 8189, CBS 8193 (BD: Guého et al. 1984); 58.6–58.9, CBS 6861, CBS 8193 (HPLC: Sugita et al. 1994).

Origin of the strains studied: CBS 8189, sour milk, Bierberg, type strain of *Oidium gracile*; CBS 5066, frozen chicken, Barnett; CBS 6861, gut of deer (*Cervus eldisiamensis*), Saëz, type strain of *T. lutetiae*; CBS 8193, from duck (*Anas querquedula*), Saëz, type strain of *Geotrichum vanrijiae*; from swan (*Cygnus olor*) (1); from crab-eating raccoon (*Procyon cancrivorus*) (1).

Type strain: CBS 8189.

Comments: The species is mostly isolated from animal sources; the type strain of *Geotrichum gracile* came from sour milk. The species is physiologically characterized by absence of growth with D-galactose, L-rhamnose and α-methyl-D-glucoside (Guého et al. 1992b). Most strains of *T. gracile* are dry, while others are usually slimy.

110.10. *Trichosporon inkin* (Oho ex Ota) do Carmo-Sousa & van Uden (1967)

Synonyms:

Microsporum brachytonum Oho (1919) nom. prov.
Sarcinomyces inkin (Oho ex Ota) Ota (1926)
Sarcinosporon inkin (Oho ex Ota) King & Jong (1975)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, colonies are finely cerebriform, often cracking the agar medium, and 9–12 mm in diameter, with a white, farinose covering; a marginal zone is absent. Appressoria are present. Hyphae disarticulate into rectangular arthroconidia, 4×(12–20) µm (Fig. 448). Sarcinae are produced on media rich in sugar. A faint odor is produced.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	v	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	v
Melibiose	–	D-Glucitol	–
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	–	Nitrite	v
L-Arabinitol	–	0.01% Cycloheximide	+
Glucono-δ-lactone	v	0.1% Cycloheximide	v
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 37°C	+
L-Lysine	+	Growth at 42°C	v

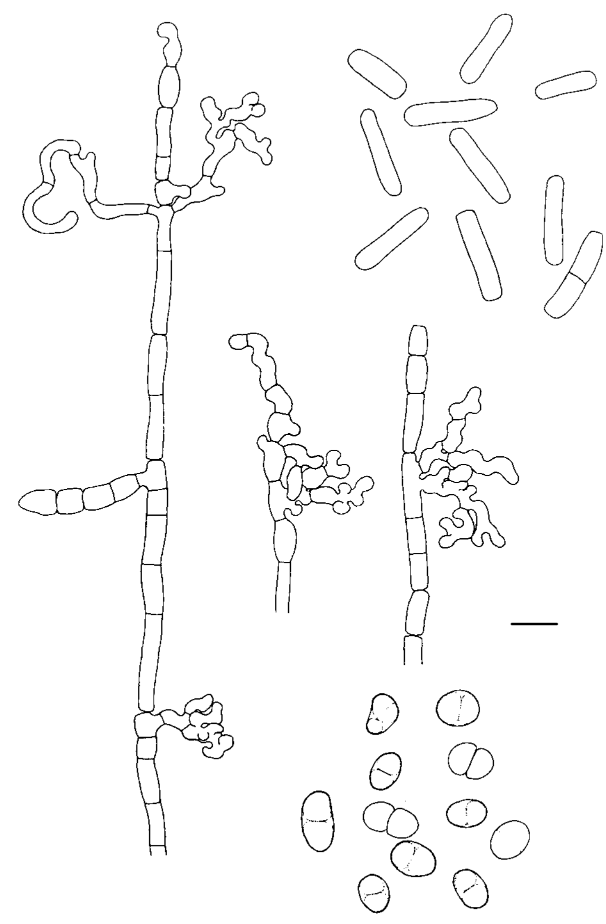


Fig. 448. *T. inkin*, CBS 5585. Disarticulating hyphae with appressoria; cylindrical arthroconidia. Slide culture with 2% malt extract agar, after 5–10 days at room temperature. Sarcinae. After 4-5 days on 50% GYA at room temperature. Bar = 10 µm.

Co-Q: 9, CBS 5585 and 4 additional strains (Guého et al. 1992b).
Mol% G+C: 58.3–59.5, CBS 5585 and 19 additional strains (T_m: Guého et al. 1992b); 61.5, CBS 5585 (BD: Guého et al. 1984).

Origin of the strains studied: CBS 5585, tinea cruris, do Carmo-Sousa; CBS 7613, anus, Stenderup; CBS 7628, endocarditis; CBS 7629, urine; pubic white piedra (8), peritonitis (1); skin lesion (1).

Type strain: CBS 5585.

Comments: On media with high sugar contents, the species produces sarcinae, and King and Jong (1975) used this property to define the genus *Sarcinosporon*. However, close molecular similarity was found between *T. inkin, T. ovoides* and *T. asahii* (Guého et al. 1992b). A diagnostic set of physiological criteria of *T. inkin* is formed from growth with α-methyl-D-glucoside, erythritol and inositol and absence of growth with L-rhamnose and melibiose. The species is ecologically remarkable because it is restricted to humans and it frequently causes cruric white piedra.

110.11. *Trichosporon jirovecii* Frágner (1969)

Synonyms:
Trichosporon cutaneum (de Beurmann, Gougerot & Vaucher) Ota var. *jirovecii* (Frágner) Frágner (1970)
Trichosporon beemeri Kuttin & Müller (1981)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are whitish, mat, cerebriform at the center with broad, flat margins with narrow but deep radial fissures, and measure 15–16 mm in diameter. Cultures have a faint odor. Hyphae are sterile or disarticulate into irregular arthroconidia, (3–5)×(6–20) µm. Endoconidia and chlamydospores (5×6 µm) are occasionally present (Fig. 449). Groups of obovoidal, sometimes catenulate conidia form in small clusters laterally at disarticulating hyphae.

Fermentation: absent.

Assimilation:

Glucose	+	N-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

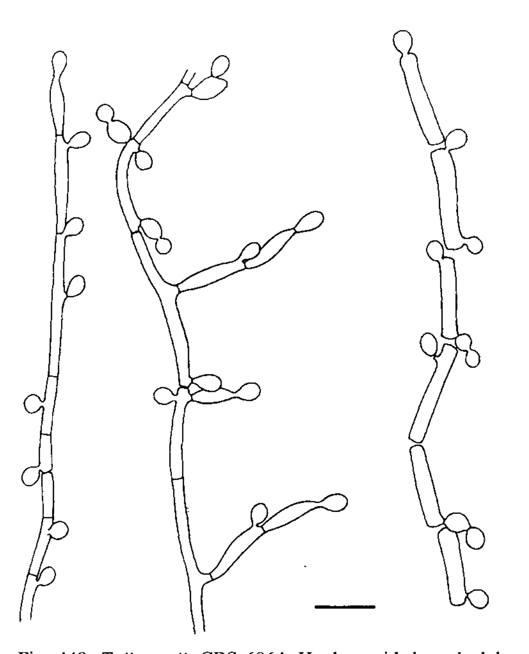


Fig. 449. *T. jirovecii*, CBS 6864. Hyphae with lateral globose conidia; disarticulating hypha. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 µm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	–
L-Arabinitol	+	0.01% Cycloheximide	+
Glucono-δ-lactone	–	0.1% Cycloheximide	v
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	v
L-Lysine	+	Growth at 37°C	–

Co-Q: 10, CBS 6864 and CBS 6950 (Guého et al. 1992b).
Mol% G+C: 61.0–61.7, CBS 6864 and CBS 6950 (T_m: Guého et al. 1992b).

Origin of the strains studied: CBS 6864, human nail, Frágner, type strain of *T. jirovecii*; CBS 6950, Nile crocodile, Kuttin, type strain of *T. beemeri*.

Type strain: CBS 6864.

Comments: The species differs from *T. mucoides* by the absence of growth with glucono-δ-lactone and from *T. moniliiforme* and *T. cutaneum* by growth with galactitol.

110.12. *Trichosporon laibachii* (Windisch) Guého & M.Th. Smith (Guého et al. 1992b)

Synonyms:
?*Trichosporon multisporum* Cochet (1940) nom. nud.
?*Trichosporon cutaneum* (de Beurmann, Gougerot & Vaucher) Ota var. *multisporum* (Cochet) Lodder & Kreger-van Rij (1952)
Endomyces laibachii Windisch (1965)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are white, mat, dry, finely farinose, and with sharp radial fissures that cause lifting of the agar; colonies are 42–46 mm in diameter. Hyphae form lateral, spheroidal conidia that are 4 μm in diameter (Fig. 450).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	v
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	v
Melibiose	+	D-Glucitol	v
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

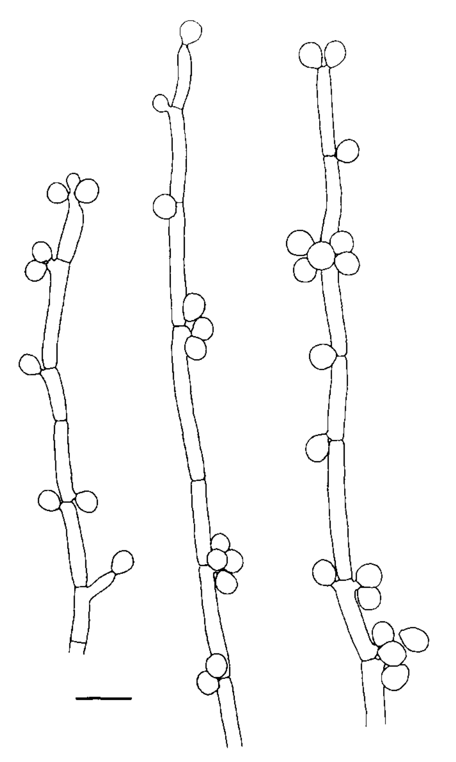


Fig. 450. *T. laibachii*, CBS 5790. Hyphae with lateral globose conidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	v	Nitrite	v
L-Arabinitol	v	0.01% Cycloheximide	+
Glucono-δ-lactone	v	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	+	Growth at 35°C	–
L-Lysine	+		

Co-Q: 9, CBS 5790 and 3 additional strains (Hara et al. 1989, Guého et al. 1992b, Sugita et al. 1994).
Mol% G+C: 57.0–57.5, CBS 5790, CBS 2495 and 2 additional strains (T_m: Guého et al. 1992b); 58.8, CBS 2495 (T_m: Guého et al. 1992b); 58.3, CBS 5790 (HPLC: Sugita et al. 1994).

Origin of the strains studied: CBS 5790, soil, Windisch, type of *Endomyces laibachii*; CBS 2495, rat droppings, Cochet, authentic strain of *T. multisporum*; water (1), soil (2).

Type strain: CBS 5790.

Comments: Judging from DNA reassociation values between 53 and 73%, *T. laibachii* and *T. loubieri* are closely related, possibly at the level of varieties (Guého et al. 1992b, Sugita et al. 1994). The taxa can readily be distinguished on the basis of micromorphology and physiology. Fusiform macroconidia are absent from *T. laibachii,* while small, lateral, ellipsoidal to spheroidal

blastoconidia are present. In addition, *T. laibachii* is able to grow with galactitol, but it fails to grow at 42°C.

The authentic strain of *T. multisporum*, CBS 2495 is close to *T. laibachii* from molecular comparisons (Guého et al. 1992b). The taxa nevertheless differ by growth responses to ribitol and creatinine and by the presence of a strong, cauliflower-like scent in cultures of *T. multisporum*.

110.13. *Trichosporon loubieri* (Morenz) Weijman (1979b)

Synonym:
Geotrichum loubieri Morenz (1963)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are white, mat, dry, finely farinose, and with sharp radial fissures that cause lifting of the agar. Colonies are 42–46 mm in diameter. Arthroconidia are present and measure (3–4)×(6–15) μm. In addition to arthroconidia, lateral or intercalary fusiform cells with granular contents are present and measure about 80×15 μm (Fig. 451).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	v	Nitrite	–
L-Arabinitol	+	0.01% Cycloheximide	+
Glucono-δ-lactone	+	0.1% Cycloheximide	+
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 37°C	+
L-Lysine	+	Growth at 42°C	+

Co-Q: 9, CBS 7065, CBS 7132 (Hara et al. 1989, Guého et al. 1992b, Sugita et al. 1994).

Mol% G+C: 56.6–56.8, CBS 7065, CBS 7132 (T_m: Guého et al. 1992b); 58.4, CBS 7065 (BD: Guého et al. 1984); 57.5, CBS 7065 (HPLC: Sugita et al. 1994).

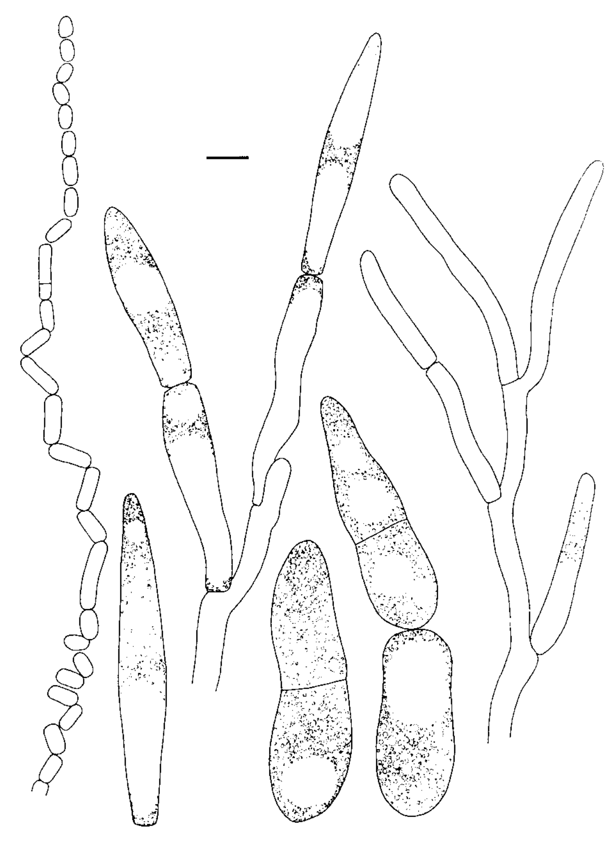


Fig. 451. *T. loubieri*, CBS 7065. Hypha disarticulating into arthroconidia; fusiform giant cells; branched hypha. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Origin of the strains studied: CBS 7065, cow mastitis, Morenz; CBS 7132, mushroom (*Entyloma* sp.), de Hoog.

Type strain: CBS 7065.

Comments: The taxon is characterized by growth at 42°C and assimilation of L-rhamnose. In addition, large, fusiform cells with granular contents are present.

110.14. *Trichosporon moniliiforme* (Weigmann & Wolff) Guého & M.Th. Smith (Guého et al. 1992b)

Synonyms:
Oidium moniliiforme Weigmann & Wolff (1909)
Oospora moniliiformis (Weigmann & Wolff) Berkhout (1923)
Trichosporon cutaneum (de Beurmann, Gougerot & Vaucher) Ota var. *antarcticum* Goto, Sugiyama & Iizuka (1969)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are cream-colored, slimy at first, but later often dry, tough and with deep but narrow, radial folds. A faint odor is exuded. Arthroconidia are 2 μm wide and ellipsoidal; lateral conidia are present and measure 2.5×4 μm (Fig. 452).

Fermentation: absent.

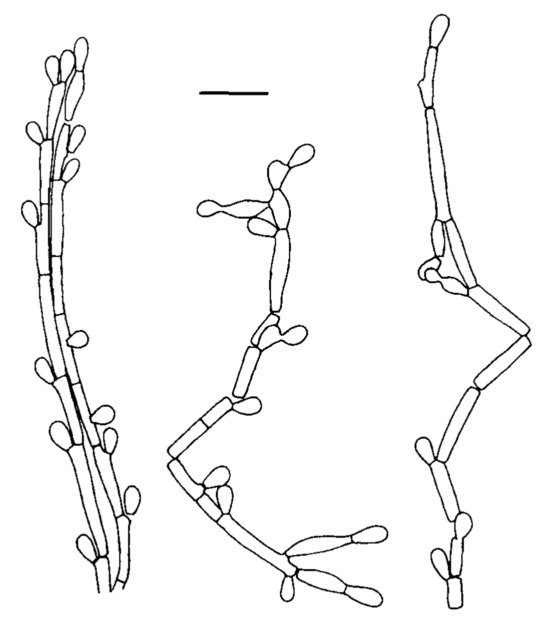


Fig. 452. *T. moniliiforme*, CBS 2467. Hyphae in small fascicles, with lateral, clavate conidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	v
L-Arabinitol	+	0.01% Cycloheximide	+
Glucono-δ-lactone	v	0.1% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	v
L-Lysine	+	Growth at 37°C	–

Co-Q: 10, CBS 2467, CBS 5959 and 5 additional strains (Hara et al. 1989, Guého et al. 1992b).

Mol% G + C: 57.8–59.0, CBS 2467, CBS 5959 and 7 additional strains (T_m: Guého et al. 1992b); 60.2, CBS 5959 (BD: Guého et al. 1984).

Origin of the strains studied: CBS 2467, butter, Bierberg; CBS 5959, Lake Vanda, Sugiyama, type strain of *T. cutaneum* var. *antarcticum*; from water (1), soil (3), pigeon guano (1), chicken run (1), flour (1).

Type strain: CBS 2467.

Comments: Physiologically the species cannot be distinguished from *T. cutaneum* on standard growth tests. However, most strains of *T. moniliiforme* were isolated from soil or water, sometimes in association with bird manure, while *T. cutaneum* is particularly known from superficial infections in humans.

110.15. *Trichosporon montevideense* (Aciole de Queiroz) Guého & M.Th. Smith (Guého et al. 1992b)

Synonyms:

Endomycopsis montevidensis Aciole de Queiroz (1973)
Geotrichum robustum Fang, Yen & Yue (1966)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are whitish, mat, somewhat hispid, strongly transversely folded, centrally coarsely cerebriform, have a citron yellow reverse and measure 22–23 mm in diameter. Cultures consist of sterile hyphae or hyphae may disarticulate into irregular arthroconidia, (3–5)×(6–20) μm (Fig. 453). Endoconidia are occasionally present.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	–
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	–
L-Arabinitol	v	0.01% Cycloheximide	+
Glucono-δ-lactone	+	0.1% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 35°C	+
L-Lysine	+	Growth at 37°C	–

Co-Q: 9, CBS 6721 (Guého et al. 1992b, Sugita et al. 1994).

Mol% G + C: 55.1–55.3, CBS 6721, CBS 8261, M9456 (T_m: Guého et al. 1992b); 57.0, CBS 6721, M9456 (HPLC: Sugita et al. 1994, Nishiura et al. 1996).

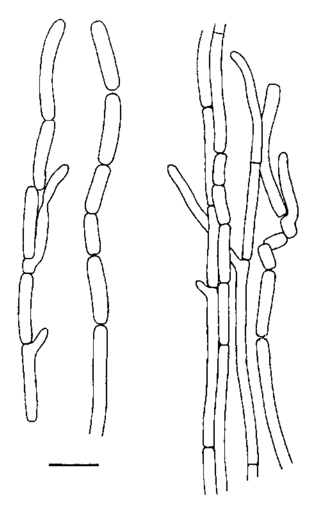


Fig. 453. *T. montevideense,* CBS 8261. Hyphae, disarticulating into arthroconidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 µm.

Origin of the strains studied: CBS 6721, water, Aciole de Queiroz; CBS 8261, human feces, Fang. M9456, SHP patient's environment, Ando.

Type strain: CBS 6721.

Comments: The strains available are rather filamentous. The species was originally described as having ascospores (Aciole de Queiroz 1973), but endoconidia may have been misinterpreted as ascospores. *Trichosporon montevideense* is physiologically recognizable by its assimilation of galactose and inability to grow with L-sorbose, L-rhamnose, melibiose and erythritol. Strain M9456 was designated as the standard for serotype II recognized within the genus (Nishiura et al. 1996).

110.16. *Trichosporon mucoides* Guého & M.Th. Smith (Guého et al. 1992b)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are cream-colored, moist and glistening, strongly elevated and become cerebriform with smooth margins, which later show narrow radial fissures. The agar adjacent to colonies often becomes cracked. Colonies measure 13–17 mm in diameter. Growth is odorless. Arthroconidia are present; shorter lateral branches often terminate with clavate conidia measuring 4×8 µm (Fig. 454).



Fig. 454. *T. mucoides,* CBS 7625. Hyphae with swollen terminal cells; liberated arthroconidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 µm.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	+
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	+	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	v
L-Arabinitol	+	0.01% Cycloheximide	+
Glucono-δ-lactone	+	0.1% Cycloheximide	v
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 37°C	+
L-Lysine	+	Growth at 42°C	–

Co-Q: 10, CBS 7625, CBS 7616 and CBS 7626 (Guého et al. 1992b).
Mol% G+C: 58.3–58.8, CBS 7625, TIMM 1573 and 4 additional strains (T_m: Guého et al. 1992b, Nishiura et al. 1996).

Origin of the strains studied: CBS 7625, meningitis, Surmont; CBS 7616, spinal fluid of human patient, Ahearn; CBS 7626, pubic white piedra (AIDS), Fischerman; CBS 7653, sputum (1); skin lesion (1); TIMM 1573, SHP patient's environment, Ando.

Type strain: CBS 7625.

Comments: The species is characterized by the combination of growth with galactitol and at 37°C. Strains of *T. faecale* may have the same combination of characters, but they are recognized by the absence of growth with L-sorbose. Most isolates are of human origin (Surmont et al. 1990). Strain TIMM 1573 was designated as the standard for serotype II recognized within the genus (Nishiura et al. 1996).

110.17. *Trichosporon ovoides* Behrend (1890)

Synonyms:

?*Pleurococcus beigelii* Küchenmeister & Rabenhorst (Rabenhorst 1867)
?*Zooglea beigeliana* Eberth 1873, cited by Redaelli & Ciferri (1941)
?*Hyalococcus beigelii* (Küchenmeister & Rabenhorst) Schroeter (1889)
?*Chlamydatomus beigelii* (Küchenmeister & Rabenhorst) Trevisan (Saccardo 1889)
?*Micrococcus beigelii* (Küchenmeister & Rabenhorst) Migula (1900)
?*Trichosporon beigelii* (Küchenmeister & Rabenhorst) Vuillemin (1902)
Geotrichum amycelicum Redaelli & Ciferri (1935)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are white, dry, farinose, irregularly folded with transverse fissures in the margins and reach 10–13 mm in diameter. Rectangular arthroconidia, (3–5)×(4–15) μm, are present (Fig. 455). Appressoria are sparingly present. No odor is detected.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	v	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	v	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	+
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	v	Citrate	v
D-Arabinose	v	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	–
D-Glucosamine	v	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	v	Nitrite	v
L-Arabinitol	–	0.01% Cycloheximide	+
Glucono-δ-lactone	v	0.1% Cycloheximide	–
Cadaverine	+	Growth at 30°C	+
Creatinine	–	Growth at 37°C	v
L-Lysine	+	Growth at 42°C	–

Co-Q: 9, CBS 7556, CBS 5585 (Guého et al. 1992b, Sugita et al. 1994).

Mol% G+C: 58.3–58.5, CBS 7556, CBS 5585 and 2 additional strains (T_m: Guého et al. 1992b); 61.7, CBS 5580 (BD: Guého et al. 1984); 60.1, CBS 7556 (HPLC: Sugita et al. 1994).

Origin of the strains studied: CBS 7556, hair white piedra, Lasagni; CBS 4098, hair, white piedra, de Arêa Leão; CBS 5580, skin lesion, Pollacci; CBS 7612 (ATCC 10266), white piedra, Miguens.

Neotype strain CBS 7556.

Comments: Two of the strains studied originated from human capital white piedra. This clinical picture corresponds well with the original description of *T. ovoides* (Behrend 1890) of which no material is known to be preserved. CBS 7556 was designated as the neotype strain of this species (Guého et al. 1992a). This name replaces the widely used but ambiguous name *T. beigelii,* for which no material nor adequate description is available.

T. ovoides is characterized by growth with erythritol and D-mannitol but not with melibiose. It shows close relationships to *T. asahii* and *T. inkin,* other *Trichosporon* species from humans that have the coenzyme Q-9 system. The three species have colonies which, when old, develop a white, farinose covering. *T. ovoides* cannot be differentiated physiologically from *T. inkin,* but DNA reassociations between the two species proved to be low (Guého et al. 1992b). *T. ovoides* develops appressoria as does *T. inkin* but has the same marginate colony as *T. asahii.*

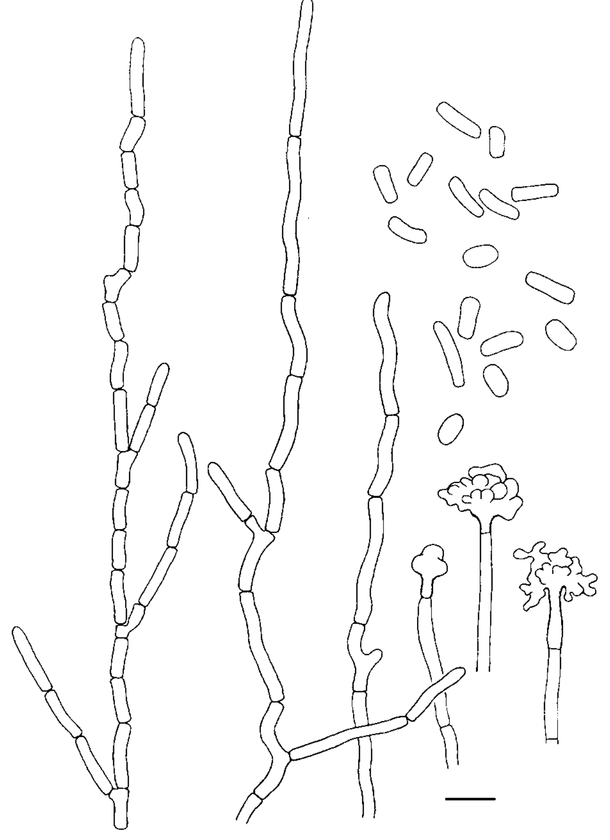


Fig. 455. *T. ovoides,* CBS 7556. Hyphae disarticulating into arthroconidia; terminal appressoria. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

110.18. *Trichosporon pullulans* (Lindner) Diddens & Lodder (1942)

Synonyms:

Oidium pullulans Lindner (1895)
Oospora pullulans (Lindner) Lindau (1907)
Oosporidium fuscans Stautz (1931)
Monilia pullulans (Lindner) Langeron & Talice (1932)
Trichosporon fuscans (Stautz) Buchwald (1939)
Basidiotrichosporon pullulans (Lindner) Kocková-Kratochvílová, Sláviková & Zemek (1977b)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are flat, soft, regularly zonate with a fringed margin, initially smooth and cream-colored, later with a whitish, farinose to velutinous covering which becomes tan to somewhat orange colored. Colonies are 19–21 mm in diameter. The odor is faint. Initial budding is enteroblastic, but in older cultures disarticulating hyphae are prevalent, and become ovoidal, or spheroidal to ellipsoidal and measure 9×(9–20) μm (Fig. 456).

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	v	Ethanol	+
Sucrose	+	Glycerol	v
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	v	Salicin	v
Inulin	–	D-Gluconate	+
Soluble starch	+	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	v	Inositol	+
D-Ribose	v	Hexadecane	n
L-Rhamnose	v	Nitrate	+
D-Glucosamine	–	Vitamin-free	v

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	v
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	+	Nitrite	+
L-Arabinitol	–	0.01% Cycloheximide	–
Glucono-δ-lactone	+	0.1% Cycloheximide	–
Cadaverine	v	Growth at 25°C	v
Creatinine	–	Growth at 30°C	–
L-Lysine	v		

Co-Q: 9, CBS 2532, CBS 2535 and CBS 2540 (Hara et al. 1989, Guého et al. 1992b); CBS 2532, CBS 2533 (Yamada et al. 1982).

Mol% G+C: 56.6–57.3, CBS 2532, CBS 2535 and 4 additional strains (T_m: Guého et al. 1992b); 58.9–59.0, CBS 2540, CBS 2542 (BD: Guého et al. 1984).

Origin of the strains studied: CBS 2532, air, Lindner; CBS 2535, slime flux of chestnut (*Castanea* sp.), Stautz, type of *Oidium fuscans*; beer pipe, (1); sawdust (1); frozen beef (1); butter (1); slime flux of maple (*Acer* sp.) (1); Antarctic soil (3); nail (1).

Type strain: CBS 2532.

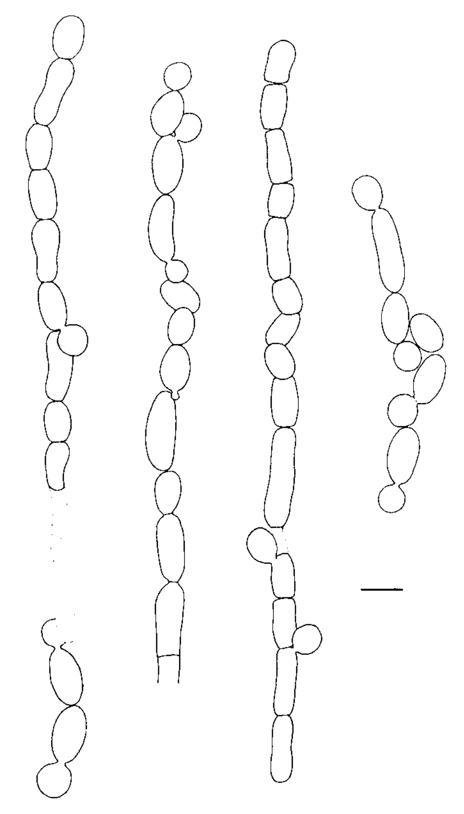


Fig. 456. *T. pullulans*, CBS 2532. Hyphae with lateral, spherical conidia. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Comments: *T. pullulans* is easily recognized by its low optimum temperature for growth (17°C), assimilation of nitrate and absence of growth with lactate. Septal pores seem to be absent (Guého et al. 1992b). Another character uncommon in the genus *Trichosporon* is enteroblastic budding. *T. pullulans* is phylogenetically distinct from the remaining *Trichosporon* species (Guého et al. 1992b).

110.19. *Trichosporon sporotrichoides* (van Oorschot) van Oorschot & de Hoog (1981)[1]

Synonym:

Trichosporiella sporotrichoides van Oorschot (1980)

Growth on Sabouraud's glucose agar: After 10 days at 20–22°C, the colonies are cream-colored, dry, mat, finely folded at the center, with a broad, flat outer zone with a fimbriate margin and are 10 mm in diameter. Cultures exude a strong, cheese-like smell. Hyphae disarticulate into elongated arthroconidia and form spheroidal lateral conidia that measure 3.5 μm in diameter (Fig. 457).

[1] Editors' Comment: Guého et al. (1992b) incorrectly listed CBS 8245 as the type strain of *Trichosporiella sporotrichoides* and subsequently followed that listing in the above treatment. According to van Oorschot and de Hoog (1981), the type strain is CBS 671.74 (CBS 8246) collected by J.H. van Emden from soil in Surinam. However, CBS 8245 (CBS 577.77) is an additional strain studied by van Oorschot and de Hoog (1981). Based on nDNA reassociation values, Guého et al. (1992b) found that CBS 8246 (the type strain of *Trichosporiella sporotrichoides*) is a synonym of *Trichosporon laibachii.* They also reported, based on LSU rRNA sequence analysis, that CBS 8245 is a distinct species within the genus, a result confirmed by Fell and Scorzetti (unpublished). Consequently it is the editors' opinion that the above description of CBS 8245 represents a new species and not *T. sporotrichoides,* which is a synonym of *T. laibachii.* This observation came to the attention of the editors late in the editing process, therefore the required revisions were not made.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	+
Galactose	+	Methanol	−
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	−
Cellobiose	+	Ribitol	−
Trehalose	+	Galactitol	−
Lactose	+	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	−	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	−	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	−	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	−
D-Glucosamine	+	Vitamin-free	−

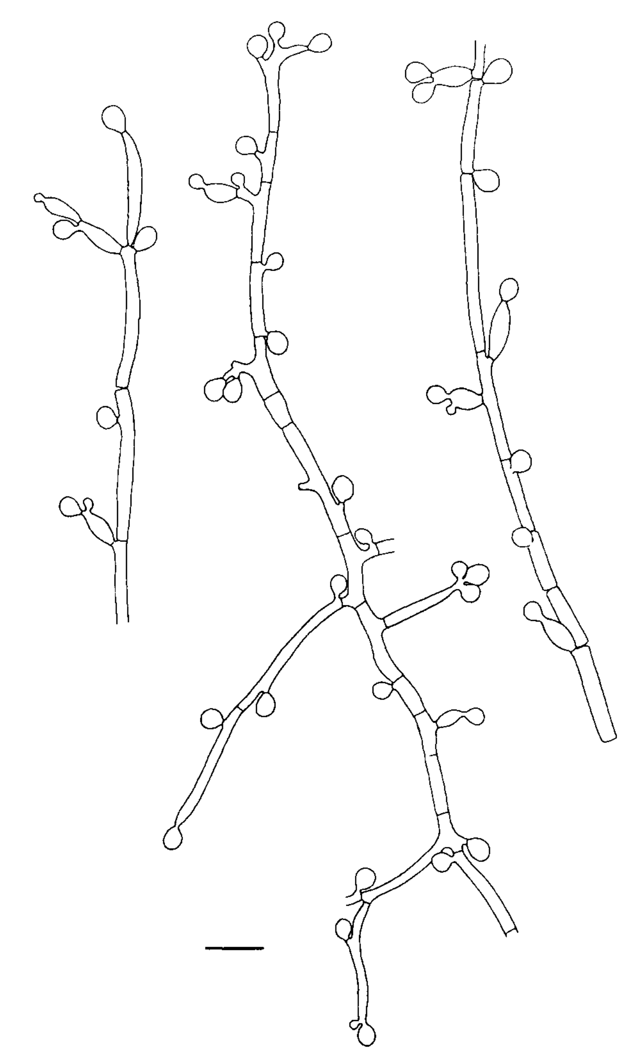


Fig. 457. *T. sporotrichoides*, CBS 8245. Hyphae disarticulating into arthroconidia which swell and may bud further. Slide culture with 2% malt extract agar, after 1–2 days at room temperature. Bar = 10 μm.

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	+	Ethylamine	+
5-Keto-D-gluconate	+	50% Glucose	+
Glucuronate	+	10% NaCl/5% glucose	+
Arbutin	+	Urease	+
Xylitol	−	Nitrite	+
L-Arabinitol	+	0.01% Cycloheximide	−
Glucono-δ-lactone	+	0.1% Cycloheximide	−
Cadaverine	+	Growth at 25°C	+
Creatinine	+	Growth at 30°C	−
L-Lysine	+		

Co-Q: 9, CBS 8245 (Guého et al. 1992b).
Mol% G+C: 61.5, CBS 8245 (T_m: Guého et al. 1992b).

Origin of the strain studied: CBS 8245, soil, Colombia.

Type strain: CBS 8245.

Comments: CBS 8245 has characteristic lateral conidia and is physiologically recognized by growth with creatinine but not with raffinose. It is similar to *T. laibachii* but distinguishable by absence of growth with raffinose and galactitol.

Comments on the genus

In the older literature, *Trichosporon* was defined largely on the basis of morphological criteria, but the genus has been redefined to comprise only arthroconidial species with a basidiomycetous affinity. rRNA sequencing studies have shown, that the genus is relatively close to *Cryptococcus* (Guého et al. 1993). The two genera probably belong to the Tremellales. In addition to a positive diazonium blue B staining reaction, presence of urease and absence of fermentation, the species have variously shaped septal dolipores. In *Trichosporon,* budding cells are sparse or absent. Some species are filamentous and produce lateral blastoconidia in addition to or occasionally instead of arthroconidia. Such strains have frequently been identified as hyphomycetes. The hyphomycete genus *Hyalodendron* is probably a synonym of *Trichosporon,* as it clustered amidst *Trichosporon* species when compared from partial 26S rRNA sequences (Guého et al. 1993).

Species of ascomycetous affinity are no longer maintained in *Trichosporon* but have been transferred to *Geotrichum.* The following three species were described by Lu and Li (1991) as members of *Trichosporon* but are excluded from the genus due to their ascomycetous affinity:

Trichosporon bacangense Lu & Li (reidentified as *Saccharomycopsis fibuliger*);
Trichosporon beijingense Lu & Li (reidentified as *Candida fennica*);
Trichosporon sinense Lu & Li (reidentified as *Candida* species).

The genus *Trichosporon* was recently revised using a combination of physiological and molecular criteria (Guého et al. 1992a,b); a review of the clinically significant species was given by Guého et al. (1994). The genus was found to consist of a large number of closely

related species, each with rather specialized ecological requirements. The concept of a limited number of extremely variable species, as maintained in the previous edition of "The Yeasts", was thus refuted. In contrast, the genus *Trichosporon* now represents a natural grouping of arthrosporous anamorphs of heterobasidiomycetes, among which are likely to be many new species that will require characterization (Sugita et al. 1995).

Older taxa for which no type material is known to exist could not be compared in this treatment and are listed below as doubtful taxa:

Endomyces rotundus Castellani (1912b)
Monilia rotunda (Castellani) Castellani & Chalmers (1913)
Oidium rotundatum (Castellani) Castellani & Chalmers (1919)
Oospora rotundata (Castellani) Berkhout (1923)
Mycoderma rotundatum (Castellani) Brumpt (1927)
Myceloblastanon rotundatum (Castellani) Ota (1928)
Geotrichum rotundatum (Castellani) Ciferri & Redaelli (1929)
Candida rotundata (Castellani) Basgal (1931)
Trichosporon rotundatum (Castellani) Puntoni (1935)
Endomyces rugosus Castellani (1912b) [nec *Mycoderma rugosa* Anderson (1917); nec *Candida rugosa* (Anderson) Diddens & Lodder (1942)]
Monilia rugosa (Castellani) Castellani & Chalmers (1913)
Hemispora rugosa (Castellani) Castellani & Chalmers (1919)
Parendomyces rugosus (Castellani) Ota (1924a)
Trichosporon rugosum (Castellani) Ota (1926)
Geotrichum rugosum (Castellani) Dodge (1935)
Geotrichum hirtum Windisch (1952) nom. nud.
Geotrichum rotundatum (Castellani) Ciferri & Redaelli var. *gallicum* Castellani (1940) nom. nud.
Hemispora pararugosa Castellani, Douglas & Thompson (1921)
Trichosporon pararugosum (Castellani, Douglas & Thompson) Nannizzi (1934)
Mycoderma pararugosa (Castellani, Douglas & Thompson) Dodge (1935)
Mycotorula mucinosa Goidànich, Ciferri & Redaelli (1939)
Neogeotrichum pulmoneum de Magalhães (1932)
Oidium brasiliense de Magalhães (1918) [nec *Zymonema brasiliense* Splendore (1912) = *Coccidioides brasiliensis* (Splendore) de Almeida (1929) = *Paracoccidioides brasiliensis* (Splendore) de Almeida (1930) = *Monilia brasiliensis* (Splendore) Vuillemin (1931)]
Geotrichum brasiliense (de Magalhães) Ciferri & Redaelli (1929)
Mycoderma brasiliense (de Magalhães) Neveu-Lemaire (1921)
Monilia brasiliensis (de Magalhães) Brumpt (1922)
Myceloblastanon brasiliense (de Magalhães) Ota (1928)
Candida brasiliensis (de Magalhães) Basgal (1931)
Trichosporon brasiliense (de Magalhães) Puntoni (1935)
Oidium pulmoneum de Magalhães (1914) [nec *Oidium pulmoneum* Bennett (1842); *Oospora pulmonea* (Bennett) Saccardo (1886) = *Mycoderma pulmoneum* (Bennett) Vuillemin (Brumpt 1927) = *Monilia pulmonea* (Bennett) Castellani & Chalmers (1913) = *Geotrichum pulmoneum* (Bennett) Basgal (1931)]
Oospora cerebriformis Kambayashi (1923)
Trichosporon cerebriforme (Kambayashi) Ota (1928)
Oospora granulosa Kambayashi (1923)
Trichosporon granulosum (Kambayashi) Ota (1928)
Parendomyces balzeri Gougerot & Burnier (Balzer et al. 1912)
Saccharomyces balzeri (Gougerot & Burnier) Castellani & Chalmers (1913)
Monilia balzeri (Gougerot & Burnier) Neveu-Lemaire (1921)
Trichosporon balzeri (Gougerot & Burnier) Ota (1926)
Trichosporium balzeri (Gougerot & Burnier) Bolognesi & Chiurco (1927)
Geotrichoides balzeri (Gougerot & Burnier) Langeron & Talice (1932)
Candida balzeri (Gougerot & Burnier) de Almeida (1933)
Proteomyces balzeri (Gougerot & Burnier) Dodge (1935)
Proteomyces variabilis Boedijn & Verbunt (1938)
Trichosporon aneurinolyticum Yonezawa, Aoki, Ota, Nishi & Matsumoto (1957) nom. nud.
Trichosporon cutaneum (de Beurmann, Gougerot & Vaucher) Ota var. *curvatum* Ohara & Nonomura (1954e) nom. nud.
Trichosporon equinum Fambach (1926)
Trichosporon hortai Niño (1933) [nec *Trichosporon hortai* Brumpt (1913)]
Trichosporon humahuaquensis Mazza & Niño (1933)
Trichosporon pardi Fernandez-Baquero & Trespalacios (1959) nom. nud.
Trichosporon ribeiroi Gomes de Moraes (1941) nom. nud.
Trichosporon undulatum Windisch (1952) nom. nud.

Species received too late for inclusion in this treatment:
Trichosporon rugosum (Castellani) Ota (1926);
Trichosporon pararugosum (Castellani, Douglas & Thompson) Nannizzi (1934);
Trichosporon rotundatum (Castellani) Puntoni (1935).

The preceding three taxa (see above for synonyms) were obtained by the courtesy of W.I. Golubev (Russian Collection of Microorganisms, Pushchino, Russia). They have the same rRNA sequence giving the priority to the specific epithet *rugosum*. Phylogenetically, the species *T. rugosum* is related to *T. ovoides* and *T. asahii.*

Recent species not available for inclusion in this treatment: *Trichosporon domesticum* Sugita, Nishikawa & Shinoda (1995)

111. *Trichosporonoides* Haskins & Spencer

G.S. de Hoog and M.Th. Smith

Diagnosis of the genus

Colonies are smooth or cerebriform, cream-colored turning to olivaceous brown. Budding is multilateral. When budding cells are formed on true hyphae or are loose, they frequently cohere in pseudomycelial chains. Chlamydospores are absent. Cell walls are multilamellar. Septal dolipore is present.

Glucose is fermented. Nitrate is assimilated. Coenzyme Q-9. Xylose and fucose are absent from cell walls. Diazonium blue B is positive. Urease is positive.

Type species

Trichosporonoides oedocephalis Haskins & Spencer

Species accepted

1. *Trichosporonoides madida* de Hoog (1979)
2. *Trichosporonoides megachiliensis* Inglis & Sigler (1992)
3. *Trichosporonoides nigrescens* Hocking & Pitt (1981)
4. *Trichosporonoides oedocephalis* Haskins & Spencer (1967)
5. *Trichosporonoides spathulata* de Hoog (1979)

Key to species

See Table 96.

1.	a Raffinose assimilated	*T. spathulata*:	p. 876
	b Raffinose not assimilated → 2		
2(1).	a Sucrose assimilated → 3		
	b Sucrose not assimilated	*T. nigrescens*:	p. 875
3(2).	a D-Xylose assimilated	*T. madida*:	p. 873
	b D-Xylose not assimilated → 4		
4(3).	a Galactose fermented	*T. oedocephalis*:	p. 875
	b Galactose not fermented	*T. megachiliensis*:	p. 874

Table 96
Key characters of species in the genus *Trichosporonoides*

Species	Fermentation		Assimilation						Growth	
	Galactose	Sucrose	Sucrose	Raffinose	Melezitose	D-Xylose	Salicin	Lactose	Vit-free[a]	37°C
Trichosporonoides madida	−	+	+	−	−	+	−	−	+	+
T. megachiliensis	−	+	+	−	−	−	−	−	n	+
T. nigrescens	+	−	−	−	−	−	−	−	+	−
T. oedocephalis	+	+	+	−	−	−	−	−	−	+
T. spathulata	+	+	+	+	+	v	+	v	+	+

[a] In vitamin-free medium.

Systematic discussion of the species

111.1. *Trichosporonoides madida* de Hoog (1979a)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 4–6 mm in diameter, cerebriform, initially mucoid, soft, with a sharp, irregular margin, cream-colored, and finally grayish-brown. Budding cells are subhyaline, ellipsoidal, (2.8–4.5)×(4.5–8.0) μm, with multilateral budding, and may cohere in short pseudomycelial chains; hyphae are mostly absent (Fig. 458).

Fermentation:

Glucose	+	Maltose	+
Galactose	−	Lactose	−
Sucrose	+	Raffinose	−

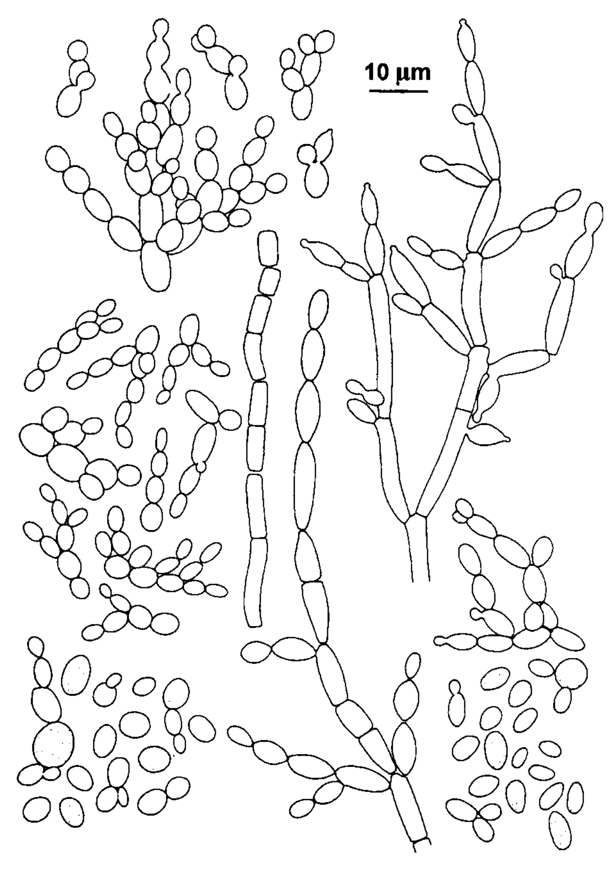


Fig. 458. *T. madida*, CBS 240.79. Yeast phase and pseudomycelium with blasto- and arthroconidia. ChA, 22°C, 5 days.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	n
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

Urease	+	Growth at 37°C	+

Co-Q: 9, CBS 240.79.
Mol% G+C: 50.2, CBS 240.79 (T_m: de Hoog and Guého 1984).

Origin of the strain studied: CBS 240.79, isolated at a margarine factory.

Type strain: CBS 240.79.

Comments: Although the source of isolation is not exactly known, *T. madida* may be regarded as a lipophilic species, as are *Moniliella suaveolens* and *Trichosporonoides spathulata.* It differs by a considerably lower mol% G+C content of DNA (de Hoog and Guého 1984). It can be differentiated from *M. suaveolens* by growth at 37°C and from *T. spathulata* by a large number of growth characteristics.

111.2. *Trichosporonoides megachiliensis* Inglis & Sigler (Inglis et al. 1992)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 14–16 mm in diameter, finely cerebriform, initially mucoid, soft, with a sharp, irregular margin, and a yellowish-brown center. Budding cells are subhyaline, ovoidal or subspherical, (4–7.5)×(4–7) µm, with multilateral budding; cells cohere in short pseudomycelial chains which separate into arthroconidia (Fig. 459). Hyphae are mostly scant.

Fermentation:

Glucose	+	Maltose	+
Galactose	–	Lactose	–
Sucrose	+	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	v
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	n
Soluble starch	v	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	v	Hexadecane	n
L-Rhamnose	–	Nitrate	v
D-Glucosamine	n	Vitamin-free	n

Additional assimilation tests and other growth characteristics:

Cadaverine	+	60% Glucose	+
Ethylamine	v	Growth at 37°C	+
L-Lysine	+		

Co-Q: 9, CBS 191.92.
Mol% G+C: Not determined.

Origin of the strains studied: CBS 190.92 (ATCC 76718), from *Ascosphaera*-infected larva of the alfalfa leafcutting bee (*Megachile rotundata*), Canada, Goettel, and two further strains from the same source.

Type strain: CBS 190.92, ex type.

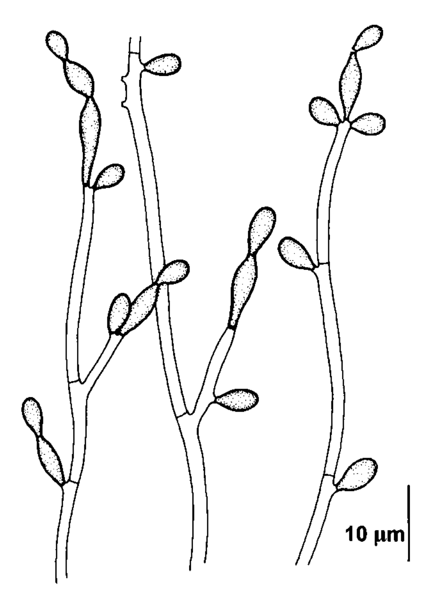


Fig. 459. *T. megachiliensis,* CBS 190.92. Mycelium with blastoconidia. PCA, 22°C, 5 days.

111.3. *Trichosporonoides nigrescens* Hocking & Pitt (1981)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 3 mm in diameter, dry, hemispherical, mustard yellow, and later cerebriform and blackish-brown. Hyphae, when present, are subhyaline and disarticulate into arthroconidia; terminally they change into pseudomycelium and produce chains of blastoconidia. Conidia are subhyaline, but become dark olivaceous, (4.0–6.5)×(5.0–8.0) µm (Fig. 460).

Fermentation:

Glucose	+	Maltose	+
Galactose	+	Lactose	–
Sucrose	–	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	n
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	+

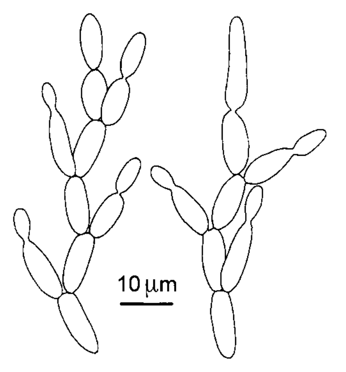


Fig. 460. *T. nigrescens,* CBS 269.81. Pseudomycelium of yeastlike phase. MEYA, 22°C, 10 days.

Additional assimilation tests and other growth characteristics:

Growth at 37°C –
Co-Q: 9, CBS 269.81.
Mol% G+C: 57, 2 strains (BD: Hocking and Pitt 1981).

Origin of the strains studied: CBS 269.81, from spoiled melon jam, Pitt, Australia; from marmalade (1).
Type strain: CBS 269.81.

Comments: The black and mustard yellow pigments rapidly disappear from cultures maintained on malt agar. Cultures are then strongly reminiscent of several *Moniliella* species. *T. nigrescens* may be a yeastlike form of the osmophilic species *Moniliella mellis* (Fabian & Quinet) V. Rao & de Hoog, which also is unable to grow with sucrose. *T. nigrescens* differs by growth with D-ribose and without vitamins.

111.4. *Trichosporonoides oedocephalis* Haskins & Spencer (1967)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 4 mm in diameter, slimy, slightly wrinkled, initially white, but soon becoming olivaceous-brown with an ochraceous reverse. Odor is strongly yeastlike. Hyphae are submerged, hyaline, (2.0–3.5) µm wide, and disarticulate into rectangular arthroconidia. Blastoconidia, which are formed in chains on hyphae, are ellipsoidal, (2.0–3.5)×(4–8) µm, and reproduce further by budding. Inflated hyphal heads that bear spherical, thick-walled, golden brown, synchronously produced conidia may also be present (Fig. 461).

Fermentation:

Glucose	+	Maltose	+
Galactose	+	Lactose	–
Sucrose	+	Raffinose	–

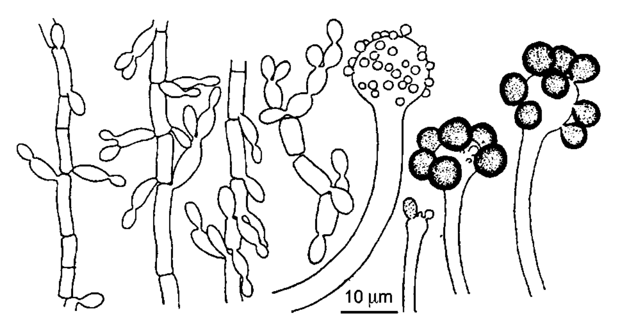


Fig. 461. *T. oedocephalis*, CBS 649.66. Pseudomycelium with blasto- and arthroconidia; swollen capitate cells with synchronous conidia. MEYA, 22°C, 30 days.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	–	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	n
Soluble starch	–	DL-Lactate	–
D-Xylose	–	Succinate	v
L-Arabinose	v	Citrate	v
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

Urease	+	Growth at 37°C	+

Co-Q: 9, CBS 649.66.
Mol% G+C: 45.8, CBS 649.66 (T_m: de Hoog and Guého 1984).

Origin of the strain studied: CBS 649.66 (ATCC 16958), from the brood of a honey bee, Haskins, California.

Type strain: CBS 649.66.

Comments: The species is close to *Moniliella mellis* (Fabian & Quinet) V. Rao & de Hoog and *M. pollinis* (Hennebert & Verachtert) de Hoog & Guého, all of which are associated with honey bees. However, the mol% G+C contents of DNA of all three species are markedly different (de Hoog and Guého 1984). *M. mellis* is recognized by its absence of growth with sucrose, while *M. pollinis* is unable to grow with galactose. The equally osmophilic species *Trichosporonoides nigrescens* differs by inability to ferment sucrose.

111.5. *Trichosporonoides spathulata* de Hoog (1979a)

Growth on 4% malt extract/0.5% yeast extract agar: After 10 days at 20–22°C, colonies are 4 mm in diameter, smooth to wrinkled, cream-colored to olivaceous, and with a vague marginal zone of submerged hyphae. Pseudomycelium is present. Hyphae are (2–3) µm wide, hyaline, and bear lateral and terminal chains of blastoconidia; hyphae soon disintegrate into rectangular arthroconidia (Fig. 462).

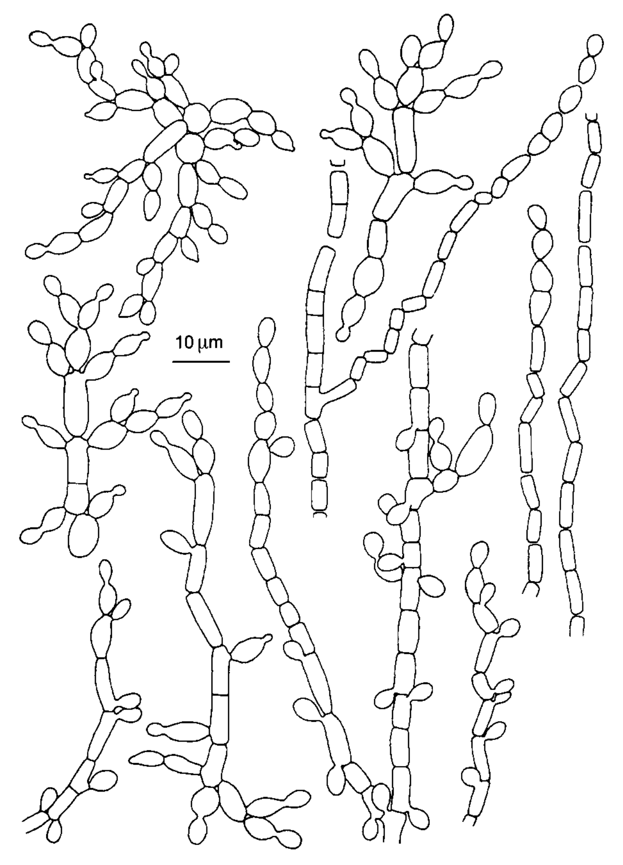


Fig. 462. *T. spathulata*, CBS 241.79. Yeastlike phase with blasto- and arthroconidia. PCA, 22°C, 5 days.

Fermentation:

Glucose	+	Maltose	+
Galactose	+	Lactose	v
Sucrose	+	Raffinose	–

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	v	Methanol	n
L-Sorbose	–	Ethanol	n
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	–
Trehalose	–	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	–	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	+	Salicin	+
Inulin	–	D-Gluconate	n
Soluble starch	–	DL-Lactate	+
D-Xylose	v	Succinate	+
L-Arabinose	–	Citrate	+
D-Arabinose	–	Inositol	–
D-Ribose	+	Hexadecane	n
L-Rhamnose	–	Nitrate	+
D-Glucosamine	n	Vitamin-free	+

Additional assimilation tests and other growth characteristics:

Urease	+	Growth at 37°C	+

Co-Q: Not determined.

Mol% G+C: 61.5–62.5, CBS 241.79, CBS 242.79 (T_m: de Hoog and Guého 1984).

Origin of the strains studied: CBS 241.79 and two additional cultures, all from ghee (buffalo milk), India.

Type strain: CBS 241.79.

Comments: The species is thus far only known from a few strains isolated from Indian buffalo milk. Its mol% G+C content is in the range of that of *Moniliella suaveolens* rather than of the remaining *Trichosporonoides* species; it differs consistently from *M. suaveolens* by its ability to grow at 37°C.

Comments on the genus

The genus is very similar to *Moniliella* Stolk & Dakin. Both genera are supposed to be of heterobasidiomycetous affinity (de Hoog 1979b), as concluded from ability to hydrolyze urea, a positive diazonium blue B staining reaction, the presence of multilamellar cell walls, dolipore septal ultrastructure (Martínez 1979), and 5S rRNA sequences (Walker 1984). Both genera are peculiar by their xerophily (Hocking and Pitt 1981) and fermentative abilities (Martínez et al. 1979), and lack xylose as well as fucose from their cell walls (Weijman 1979a). The cellular dimensions in *Moniliella* are generally larger than in *Trichosporonoides* (de Hoog 1979a, Inglis et al. 1992).

Ramírez (1989) described a *Trichosporonoides* species, *T. australiensis* Ramírez, from orange-mango drink in Australia. No culture is available for study. Inglis et al. (1992) considered *T. australiensis* to be synonymous with *Brettanomyces anomalus*.

112. *Tsuchiyaea* Y. Yamada, Kawasaki, M. Itoh, Banno & Nakase

Y. Yamada and I. Banno

Diagnosis of the genus

Cells are globose, ovoid to cylindrical, and reproduce by the formation of enteroblastic buds or of conidia on stalks. A single conidium is formed at the top of the stalks and liberated at a septum in the mid-region of the stalks without projection. Ballistoconidia are absent.

Glucose is not fermented. D-Glucuronate and *myo*-inositol are assimilated. Diazonium blue B color reaction is positive. Xylose is present in the cells. Nitrate is not assimilated. Urease is produced. Coenzyme Q-9 is present.

Type species

Tsuchiyaea wingfieldii (van der Walt, Y. Yamada & Ferreira) Y. Yamada, Kawasaki, M. Itoh, Banno & Nakase

Species accepted

1. *Tsuchiyaea wingfieldii* (van der Walt, Y. Yamada & Ferreira) Y. Yamada, Kawasaki, M. Itoh, Banno & Nakase (1988)

Systematic discussion of the species

112.1. *Tsuchiyaea wingfieldii* (van der Walt, Y. Yamada & Ferreira) Y. Yamada, Kawasaki, M. Itoh, Banno & Nakase (1988b)

Synonym:
Sterigmatomyces wingfieldii van der Walt, Y. Yamada & Ferreira (1987d)

Growth in malt extract: After 3 days at 25°C, the cells are globose to ellipsoidal, (4.0–10.0)×(3.0–9.0) μm, reproduce by enteroblastic budding, and occur singly, in pairs or short chains. Giant cells, 8.0×14.0 μm, may be present. A sediment, ring and islets are formed. After 4 weeks at ambient temperature, a sediment and a thick, wrinkled creeping pellicle are present.

Growth on malt agar: After 3 days at 25°C, the cells are globose, ellipsoidal, ovoid, occasionally apiculate, (3.0–11.0)×(3.0–10.0) μm, occur singly, in pairs or short chains, and reproduce either by enteroblastic budding or by the formation of stalks which delimit conidia terminally. The conidia are detached by disjunction at a septum in the mid-region of the stalks. The mature, detached conidia, (9.5–14.0)×(5.5–6.5) μm, are clavate to spathulate, with truncate bases. Stalk-forming cells are, as a rule, lipid-rich, and form 1–3 stalks, which measure (2.0–10.0)×(1.5–2.0) μm. The streak culture is butyrous, creamy-white, and faintly crispulate with an entire margin. After 4 weeks at ambient temperature, the culture is crispulate, cream-colored and dull with a lobate margin.

Dalmau plate culture on corn meal agar: Neither pseudomycelium nor mycelium is formed.

Fermentation: absent.

Assimilation:

Glucose	+	*N*-Acetyl-D-glucosamine	s
Galactose	+	Methanol	–
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	–	Erythritol	+
Cellobiose	+w	Ribitol	+
Trehalose	+	Galactitol	–
Lactose	v	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	+
Inulin	–	D-Gluconate	s
Soluble starch	–	DL-Lactate	–
D-Xylose	+	Succinate	v
L-Arabinose	s	Citrate	–
D-Arabinose	s	Inositol	+
D-Ribose	+	Hexadecane	–
L-Rhamnose	+	Nitrate	–
D-Glucosamine	w/–	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

2-Keto-D-gluconate	s	Urease	+
5-Keto-D-gluconate	–	D-Glucono-1,5-lactone	–
Arbutin	+	Thiamine-free	–
L-Lysine as N-source	w	Biotin-free	–
Ethylamine	–	0.01% Cycloheximide	–
50% Glucose	+	Growth at 25°C	+
60% Glucose	–	Growth at 30°C	–
Starch formation	w		

Co-Q: 9.
Mol% G+C: 55.37, type strain (CBS 7118); 53.3 (HPLC).
Xylose in cells: Present.

Origin of the strain studied: IFO 10204 (CBS 7118), frass of a Scolytid beetle in *Olea europae* subsp. *africana*, J.P. van der Walt, South Africa.

Type strain: CBS 7118 (IFO 10204).

Comments: *T. wingfieldii* is morphologically unique in reproducing either by enteroblastic budding or by the formation of conidia on the apex of stalks, and in containing xylose in the cells. The presence or absence of xylose in

cells has been utilized as an important criterion for classifying basidiomycetous yeasts at the generic level. By these characteristics *Tsuchiyaea* is distinguished from *Sterigmatomyces. T. wingfieldii* conidia are separated at the midpoint of the short stalks and the species have coenzyme Q-9. In contrast, *Fellomyces* and *Kurtzmanomyces* have conidia that are disjointed at the distal end of the stalks and have coenzyme Q-10. The latter two genera therefore are clearly discriminated from *Tsuchiyaea.* The characteristic of the type culture is that its propagation by stalked conidia appears to be limited to growth on solid media, a characteristic that is not observed in malt extract.



Part VII

Prototheca, a yeastlike alga

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113. *Prototheca* Krüger

R.S. Pore

Diagnosis of the genus

Colonies are yeastlike, but budding is absent. Reproduction is by the formation of a variable number of sporangiospores (2–16 or more) within a sporangium. The sporangiospores are released through a split in the sporangial wall and usually disperse (Fig. 463). The reproductive morphology is similar to that of *Chlorella* and is the basis for a presumptive phylogenetic relationship to the algae, however, *Prototheca* lacks plastids or vestigial plastids (referred to in the literature, but not confirmed by this author). The sporangium is nearly spherical, or an enlarged version of the sporangiospore. The sporangiospores are usually spherical or ellipsoidal, but reniform and falcate strains occur. A dauer cell stage resists staining in Rose Bengal stain[1]. No sexual stage occurs (Pore 1985).

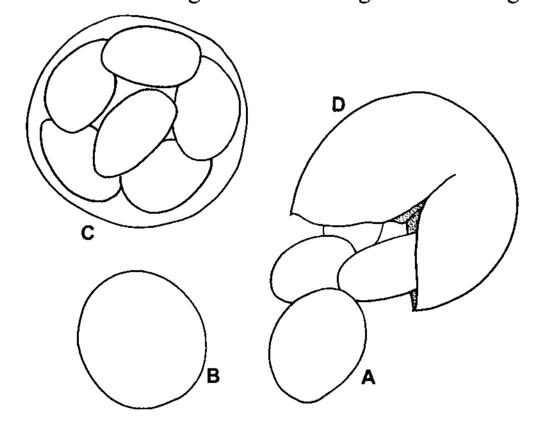


Fig. 463. Schematic life cycle of *Prototheca.* A sporangiospore (A) enlarges to form a dauer cell (B), undergoes further development to a sporangium (C), which releases more sporangiospores (D).

Ammonium chloride is assimilated as a sole nitrogen source, but nitrate is not (Pore 1972). Glucose is assimilated and the assimilation of a few other carbon substrates has taxonomic significance (Pore 1985). Lactic acid is produced from glucose by *P. zopfii* and some species produce acid, but not gas from glucose. Glycogen is the storage carbohydrate; starch is absent. Neither chlorophylls nor carotenoids are produced. Thiamine and oxygen are required for growth. The cell wall resists acid and alkaline hydrolysis (Conte and Pore 1973).

Type species

Prototheca zopfii Krüger

Species accepted

1. *Prototheca moriformis* Krüger (1894)
2. *Prototheca stagnora* (Cooke) Pore (1985)
3. *Prototheca ulmea* Pore (1986)
4. *Prototheca wickerhamii* Tubaki & Soneda (1959)
5. *Prototheca zopfii* Krüger (1894)
   a. *Prototheca zopfii* Krüger var. *zopfii* (1985)
   b. *Prototheca zopfii* var. *hydrocarbonea* Pore (1985)
   c. *Prototheca zopfii* var. *portoricensis* Pore (1985)

Key to species

See Table 97.

1.	a	Sporangiospores greater than 5.7 µm in long dimension; dauer cells greater than 8.5 µm → 2		
	b	Sporangiospores less than 4.3 µm in long dimension; dauer cells less than 8.5 µm → 3		
2(1).	a	Capsule absent upon primary isolation	*P. zopfii*:	p. 886
	b	Capsule present upon primary isolation	*P. moriformis*:	p. 884
3(1).	a	Trehalose assimilated	*P. wickerhamii*:	p. 886
	b	Trehalose not assimilated → 4		
4(3).	a	Fructose assimilated	*P. stagnora*:	p. 885
	b	Fructose not assimilated	*P. ulmea*:	p. 885

[1] Rose Bengal Stain for *Prototheca* species (Pore 1985): 100 mg Rose Bengal + 100 ml distilled water. Stain 1–10 min.; all cells stain red except the dauer cells.

Table 97
Key characters of species in the genus *Prototheca*

Species	Assimilation				Morphology			
	Fructose	Galactose	1-Propanol	Trehalose	Dauer cell <8.5 µm[a]	Dauer cell >8.5 µm[b]	Spherical[c]	Capsule[d]
Prototheca moriformis	+	–	+/w	–	–	+	v	+
P. stagnora	+	+	+/w	–	+	–	+	+
P. ulmea	–	–	–	–	+	–	v	+
P. wickerhamii	+	+	–	+	+	–	+	–
P. zopfii	+	–	+	–	–	+	v	–

[a] Dauer cell less than 8.5 µm in diameter, sporangiospores less than 4.3 µm.
[b] Dauer cell greater than 8.5 µm in diameter, sporangiospores greater than 5.7 µm.
[c] All cell stages obviously nearly spherical.
[d] Cell surrounded by clear zone when negatively stained upon primary isolation. All tests were done in *Prototheca* isolation medium at 25°C with 1% wt./vol. carbon sources (Pore 1985).

Systematic discussion of the species

113.1. *Prototheca moriformis* Krüger (1894a)

Synonym:
Prototheca moriformis Krüger var. *betulinus* Chodat (1913)

Growth on *Prototheca* isolation medium (PIM): The *Prototheca* isolation medium is described in Table 98. After 5–10 days, cells are encapsulated, resulting in a slimy, glistening, mucoid to viscoelastic, translucent to white colony. Newly released sporangiospores are spherical, or more frequently ellipsoidal, (3–5×4–6, ave. 4×5 µm). Dauer spores are usually ellipsoidal (7–9×9–11, ave. 8×10 µm). The sporangium is spheroidal or ellipsoidal, (8–15×10–20, ave. 12×14 µm). Sporangiospores rapidly developed a 1–5 µm (ave. 3 µm) thick capsule that is retained as the cell develops.

Growth on glucose-peptone based media, eg., Sabouraud's medium: The colony type remains the same, but a tan pigment accumulates with age. Cell morphology and size varies slightly depending on the substrate. Temperature optima vary between 22–30°C, but some strains grow at 37°C. *P. moriformis* grows over a wide pH range.

Fermentation: absent.

Table 98
Prototheca Isolation Medium (PIM)

Distilled water	1 L
Potassium hydrogen phthalate	10 g
Sodium hydroxide	0.9 g
Magnesium sulfate	0.1 g
Potassium phosphate, monobasic	0.2 g
Ammonium chloride	0.3 g
Thiamine	0.001 g
Purified agar	15 g
Carbon source[a]	10 g
pH	5.1±0.1
Autoclave	15 min.

[a] For assimilation test media, substitute carbon source 1% wt./vol. For isolation medium, add glucose and 5-fluorocytosine (0.25 g/L) (Pore 1973).

Assimilation (48 hours):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	n	Ethanol	n
Sucrose	–	Glycerol	v
Maltose	w	Erythritol	n
Cellobiose	n	Ribitol	n
Trehalose	–	Galactitol	n
Lactose	v	D-Mannitol	–
Melibiose	–	D-Glucitol	n
Raffinose	–	α-Methyl-D-glucoside	n
Melezitose	–	Salicin	n
Inulin	n	D-Gluconate	n
Soluble starch	–	DL-Lactate	n
D-Xylose	–	Succinate	n
L-Arabinose	–	Citrate	n
D-Arabinose	n	Inositol	–
D-Ribose	n	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	n	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

1-Propanol	+/w	Acetate (pH 5.1)	+/w
D(–)Fructose	+	Clotrimazole	v
D(+)Mannose	–		

Origin of the strains studied: Thirty-five strains were examined for morphological and major nutritional traits. Strains excluded from the species *P. moriformis* were NRRL Y-6865, Y-6867, Y-6869, and Y-6871; CDC B-1444 and B-1692; ATCC 16522, 16523 and 16524; UTEX 1434. *P. moriformis* has been isolated from plant, animal, soil, aqueous and sewage habitats (Pore 1985).

Type strain: ATCC 50081, cheese factory waste water, via R.S. Pore (1985); neotype (Pore 1985).

Comments: Krüger's (1894a,b) original description of slimy *Prototheca* sp. was confirmed by Pringsheim (1963) and Pore (1985). It was acknowledged by Pore (1985) that the main difference between *P. moriformis* and *P. zopfii* is the presence of the distinctive capsule that also causes the slimy or mucoid colony characteristic of *P. moriformis*. It was also acknowledged that a few strains of *P. moriformis* lost their capsule forming capacity in long-term culture, making them indistinguishable from *P. zopfii* by current

methods. This is a reversible phenomenon, the cause of which is unknown. The major justification for maintaining *P. moriformis* as a separate species is that typical isolates, which are stable for the capsule trait, are readily obtained from nature. The capsular polymer is a polyglycan composed primarily of galactose and rhamnose. *P. moriformis* was shown to have a DNA base composition similar to *P. zopfii*, but the DNA homology was only about 25% (Huss et al. 1988).

113.2. *Prototheca stagnora* (Cooke) Pore (1985)

Synonym:
Prototheca viscosa Pringsheim 1963 nom. nud.

Growth on PIM: After 5–10 days, cells are encapsulated, resulting in a slimy, glistening, translucent to whitish colony. Newly released sporangiospores are spherical, (2.5–4.5, ave. 3.5 μm). Dauer cells are spherical, (5.5–8, ave. 6.5 μm), as are sporangia (7–14, ave. 9.5 μm). Rarely, sporangiospores are spheroidal or ellipsoidal. All stages are encapsulated, capsules are 1–5 (ave. 3 μm) in thickness. In a few strains, the capsule characteristic was lost upon serial subculture.

In addition to PIM, *P. stagnora* grows on other media, including Sabouraud's medium. No significant differences (an exception was the development of a variable tan colony pigment in old cultures) in the colony type or cell morphology on other media were noted. Neither temperature optima nor pH is critical for growth, but 25±5°C and pH 5–8 are usually suitable.

Fermentation: absent.

Assimilation (72 hours):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	+	Methanol	n
L-Sorbose	n	Ethanol	n
Sucrose	–	Glycerol	v
Maltose	w	Erythritol	n
Cellobiose	n	Ribitol	n
Trehalose	–	Galactitol	n
Lactose	v	D-Mannitol	–
Melibiose	–	D-Glucitol	n
Raffinose	–	α-Methyl-D-glucoside	n
Melezitose	–	Salicin	n
Inulin	n	D-Gluconate	n
Soluble starch	–	DL-Lactate	n
D-Xylose	–	Succinate	+
L-Arabinose	–	Citrate	n
D-Arabinose	n	Inositol	–
D-Ribose	n	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	n	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

1-Propanol	+/w	Acetate (pH 5.1)	w/–
D(–)Fructose	+	Clotrimazole	v
D(+)Mannose	v		

Origin of the strains studied: Twenty-five strains were examined for morphological and major nutritional traits; of these 19 were directly isolated from nature. Strains from culture collections included NRRL Y-6872, ATCC 16528 and CDC B–1277. A strain excluded from the species was UTEX 1442 (ATCC 16527).

Type strain: ATCC 16528, from sludge, via W.B. Cooke.

Comments: *P. stagnora* was emended by Pore (1985) to include only the smaller of the two sizes originally described by Cooke (1968). Also, the capsule characteristic was added to the emended description, but the originally reported assimilation of sucrose was not substantiated in the emended description. Subsequently, strains of *P. stagnora* which were unable to assimilate fructose were transferred to *P. ulmea* (Pore 1986). *P. stagnora,* in order of frequency, was isolated from sewage and plant material.

113.3. *Prototheca ulmea* Pore (1986)

Growth on PIM: After 5–10 days, cells are encapsulated resulting in a nonpigmented mucoid colony. Sporangiospores are ellipsoidal to subglobose, (2.8–3.0×3.0–3.5 μm, ave. 2.9×3.3 μm). Sporangia are spheroidal, (7.2–10 μm, ave. 8.6 μm). Dauer cells are ellipsoidal, (4.5–5.8×5.5–8.0 μm, ave. 5.3× 6.7 μm). Capsule thickness is 2–5 μm, ave. (3.5 μm).

P. ulmea grows on additional laboratory media such as Sabouraud's medium and the colony and morphology is similar to that produced on PIM. Temperature optima (approx. 25°C) and pH are not critical for growth, but *P. ulmea* is the slowest growing and least robust *Prototheca* species.

Fermentation: absent.

Assimilation (72 hours):

Glucose	+	*N*-Acetyl-D-glucosamine	n
Galactose	–	Methanol	n
L-Sorbose	n	Ethanol	n
Sucrose	–	Glycerol	n
Maltose	–	Erythritol	n
Cellobiose	n	Ribitol	n
Trehalose	–	Galactitol	n
Lactose	–	D-Mannitol	–
Melibiose	–	D-Glucitol	n
Raffinose	–	α-Methyl-D-glucoside	n
Melezitose	–	Salicin	n
Inulin	n	D-Gluconate	n
Soluble starch	–	DL-Lactate	n
D-Xylose	–	Succinate	n
L-Arabinose	–	Citrate	n
D-Arabinose	n	Inositol	–
D-Ribose	n	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	n	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

1-Propanol	–	Acetate (pH 5.1)	–
D(–)Fructose	–	Clotrimazole	v
D(+)Mannose	–		

Origin of the strains studied: Eighteen strains isolated from nature were examined for morphological and major nutritional traits.

Type strain: ATCC 50112, from slime flux of elm, via R.S. Pore.

Comments: Some strains of *P. ulmea* were originally included with *P. stagnora* Pore (1985), but based upon several distinctive features, a separate species was later described (Pore 1986). *P. ulmea* was ellipsoidal and did not assimilate fructose nor any known carbohydrate other than glucose. Many strains were isolated from the slime flux of various elm species, including the American elm (*Ulmus americana*).

113.4. *Prototheca wickerhamii* Tubaki & Soneda (1959)

Growth on PIM: A white or off-white yeastlike colony forms after 5 days. All cell stages are spherical. Sporangiospores are 2.5–4.5 µm (ave. 3.2 µm), sporangia are 7–13 µm (ave. 9.4 µm), and dauer cells are 5.5–8.5 µm (ave. 6.5 µm) in diameter.

Temperature optima are usually above 25°C and some strains grow at 37°C. The pH range is wide. A wide range of media support growth. On Sabouraud's medium, for example, the colony characteristically develops a tan pigmentation with age.

Fermentation: absent.

Assimilation (48 hours):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	+	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	–	Erythritol	–
Cellobiose	–	Ribitol	n
Trehalose	+	Galactitol	n
Lactose	v	D-Mannitol	–
Melibiose	–	D-Glucitol	n
Raffinose	–	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–	Succinate	n
L-Arabinose	–	Citrate	n
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	–
L-Rhamnose	–	Nitrate	–
D-Glucosamine	n	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

1-Propanol	–	Acetate (pH 5.1)	–
D(–)Fructose	+	Clotrimazole	w/–
D(+)Mannose	v		

Origin of the strains studied: Eighty-five strains were examined for morphological and major nutritional traits. Representatives from culture collections included UTEX 1440; NRRL Y-2348, Y-6865, Y-6867, Y-6869, Y-6871, Y-7029 and Y-7045; CDC 45-1029-70, 43-1068-70, B-1280, B-1281, B-1282, B-1418, B-1444, B-1694, and B-2224; ATCC 16522, 16523, 16529 and 16531.

Type strain: ATCC 16529, from household plumbing, via W.B. Cooke.

Comments: *P. wickerhamii* is the only species for which there is a corresponding species of *Chlorella* with shared properties. *Chlorella protothecoides* Krüger (1894a,b) and *P. wickerhamii* both require thiamine and use ammonium (but not nitrate) nitrogen for growth, while most species of *Chlorella* are otherwise (Pore 1972). In addition, both species assimilate trehalose (Kessler 1982), exhibit acetate (pH 5.1) inhibition, and grow well on PIM, a selective medium. Like *Prototheca* spp., but unlike many other *Chlorella* spp., *C. protothecoides* stores glycogen and has a similar cell wall (Conte and Pore 1973).

The DNA base composition of *P. wickerhamii* was closer to that of *C. protothecoides* than were those of other *Prototheca* spp. tested, but DNA binding homologies showed great heterogeneity between the two, as well as between the *Prototheca* spp. themselves (Huss et al. 1988). Confirmation of the relationship between *P. wickerhamii* and *C. protothecoides* comes from 16S-like rRNA gene DNA sequence similarities that place these two species apart from other *Chlorella* spp. tested (Huss and Sogin 1990). Further sequence and structural analysis of the conserved SSU rRNA mitochondrial gene inferred a close phylogenetic relationship between *P. wickerhamii* and plants (Wolff and Kück 1990).

P. wickerhamii was found in high numbers in all domestic and municipal sewage where it is numerically the most common *Prototheca* sp. (Pore et al. 1986). It has been isolated from many aquatic sources, food, plants, and animals. It may be infrequently isolated from human feces, but a causative effect has not been determined. It was weakly pathogenic for mice and was the principal cause of protothecosis in man (Pore et al. 1983, Pore 1997).

113.5. *Prototheca zopfii* Krüger (1894a)

This species has three varieties:

Prototheca zopfii Krüger var. *zopfii* (1985)

Synonyms:
Prototheca zopfii Krüger (1894a)
Prototheca chlorelloides Beijerinck (1904)
Prototheca ciferrii Negroni & Blaisten (1941)
Prototheca salmonis Gentles & Bond (1977)
Prototheca seqbwema Davis, Spencer & Wakelin (1964)
Prototheca trispora Ciferri, Montemartini & Ciferri (1957)
Prototheca ubrizsyi Zsolt & Novák (1968)

Prototheca zopfii var. *hydrocarbonea* (Kocková-Kratochvílová & Havelkova) Pore (1985)

Synonym:
Prototheca hydrocarbonea Kocková-Kratochvílová & Havelkova (1974)

Prototheca zopfii var. *portoricensis* (Ashford, Ciferri & Dalmau) Pore (1985)

Synonym:
Prototheca portoricensis Ashford, Ciferri & Dalmau (1930)

Growth on PIM: After 5 days, colonies are white or off-white, creamy to distinctly roughened or wrinkled. Cells are commonly spheroidal to ellipsoidal, but a few strains

are reniform. Cells are waxy and hydrophobic. Newly released sporangiospores are spheroidal, (4.5–7.5 μm, ave. 6.5 μm), or ellipsoidal (3–7×5–8 μm, ave. 5.5×6.5 μm). Dauer cells are spheroidal, (8.5–14 μm, ave. 11 μm), or ellipsoidal (6–11×8.5–13 μm, ave. 9.5×11.5 μm), and sporangia are spheroidal, (14–25 μm, ave. 17.3 μm), or ellipsoidal (11–20×14–23 μm, ave. 14.5×16.5 μm) Fig. 464).

Cell morphology and colony type vary depending on substrate and environmental conditions. Temperature and pH variation are tolerated. Some strains grow at 37°C. *P. zopfii* grows more vigorously than other species in culture.

Fermentation: absent.

Assimilation (48 hours):

Glucose	+	*N*-Acetyl-D-glucosamine	–
Galactose	–*	Methanol	–
L-Sorbose	–	Ethanol	+
Sucrose	–	Glycerol	+
Maltose	w	Erythritol	–
Cellobiose	–	Ribitol	n
Trehalose	–	Galactitol	n
Lactose	v	D-Mannitol	–
Melibiose	–	D-Glucitol	n
Raffinose	v	α-Methyl-D-glucoside	–
Melezitose	–	Salicin	–
Inulin	–	D-Gluconate	–
Soluble starch	–	DL-Lactate	+
D-Xylose	–*	Succinate	–
L-Arabinose	–	Citrate	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Hexadecane	v
L-Rhamnose	–	Nitrate	–
D-Glucosamine	n	Vitamin-free	–

Additional assimilation tests and other growth characteristics:

1-Propanol	+	Acetate (pH 5.1)	+
D(–)Fructose	+	Clotrimazole	v
D(+)Mannose	–		

*Assimilation (w/+) on suitable basal medium in published reports, but not confirmed.

Origin of the strains belonging to the variety *zopfii*: ATCC 16524, 16532, 16533; UTEX 289, 328, 1434; NRRL Y-6868, Y-7056, Y-7059, YB-2462, YB-4121; CDC B-1268, B-1271, B-1272, B-1285, B-1692, all of unknown origin. UTEX 1442 (sewage), NRRL YB-990 and YB-4825 (plants), ATCC 16527 (sewage); CDC B1270 (animal).

Type strain: ATCC 16533.

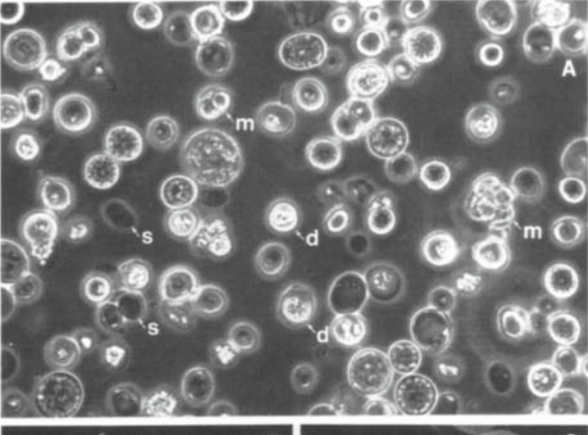
Origin of the strains belonging to the variety *hydrocarbonea*: ATCC 30253 (marine sediment), NRRL Y-7676 (origin unknown).

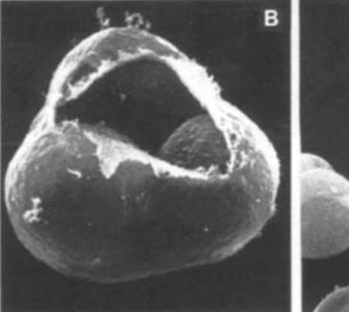
Type strain: ATCC 30253.

Origin of the strains belonging to the variety *portoricensis*: NRRL YB-4826 (human intestine), UTEX 178 (unknown).

Type strain: NRRL YB-4826.

Comments: Negroni and Blaisten (1941), and later Cooke (1968) designated *P. zopfii* as the lectotype for





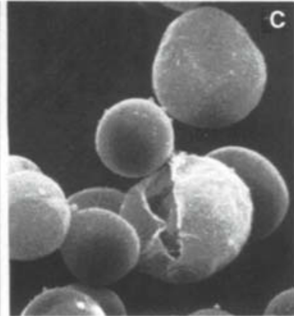


Fig. 464. *P. zopfii*. Phase contrast microscopy: (A) s, sporangiospore; d, dauer cell; m, mature sporangium. (B)–(C) Sporangium and sporangiospores by scanning electron microscopy.

Prototheca. Krüger (1894a,b) described *P. zopfii* as spheroidal, but Pore (1985) concluded that 75% of the strains were ellipsoidal, 25% were spheroidal, and a few strains were reniform. Reniform strains have been referred to as *P. zopfii* var. *portoricensis* Pore (1985), but there are no nutritional traits to otherwise distinguish this taxon. Hexadecane-assimilating strains (Walker and Pore 1978) have been referred to as *P. zopfii* var. *hydrocarbonea* Pore (1985), but there are no other nutritional or morphological traits to otherwise distinguish this taxon.

Blaschke-Hellmessen et al. (1985) reported that three unnamed varieties of *P. zopfii* differed from one another morphologically and nutritionally. Two varieties were reported to assimilate galactose but, this could not be confirmed when PIM was used as the basal medium (Pore 1985). Hence, assimilation of galactose varied depending on the basal medium used. Additional synonyms used in the literature, but for which no authorities were found are *P. crieana, P. krugeri, P. pastoriensis, P. portoricensis* var. *ciferrii, P. portoricensis* var. *trispora*, and *P. tropicalis*.

In long term culture some strains spontaneously, but reversibly, develop an extracellular polysaccharide capsule, making them morphologically and physiologically indistinguishable from *P. moriformis* (Pore 1985). *P. zopfii* is common in sewage and has been isolated from many plant and animal sources. It caused mastitis in dairy cattle (Pore et al. 1987, Pore 1997).

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Part VIII

Key to species

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Chapter 114

Key to species

R.W. Payne, C.P. Kurtzman and J.W. Fell

1. Introduction

Species given in this key are those that readily grow in the standard media used for fermentation and assimilation tests. Consequently, *Coccidiascus*, *Endomyces*, Endomycete-like species and *Malassezia* are excluded here, but are given in the keys to genera. Some species occur more than once in the key, depending on variability of fermentation and growth reactions.

Data for *Lalaria* species are limited and members of this genus are omitted from the key. However, *Lalaria* is given in the Key to the genera of ascomycetous yeasts. *Fellomyces horovitziae* is characterized in the treatment of the genus *Fellomyces*, but the data were received too late for inclusion in this key.

When sexual states are known for common anamorphic species, the taxon is given in the key under the teleomorphic name. For example, *Candida lipolytica* is given under *Yarrowia lipolytica* and *Phaffia rhodozyma* is given as *Xanthophyllomyces dendrorhous*. Known anamorph–teleomorph connections can be made by consulting the indexes.

Species separations in this key are often made from the reaction on a single test. Consequently, species descriptions need to be consulted to verify the identification.

#	Test	Positive	Negative
1	Diazonium Blue B reaction	→ 2	→ 318
2	Glucose fermentation	→ 3	→ 18
3	Erythritol growth	→ 4	→ 12
4	Sucrose growth	→ 5	→ 11
5	DL-Lactate growth	*Trichosporonoides spathulata*: p. 876	→ 6
6	Growth at 37°C	→ 7	*Moniliella suaveolens*: p. 787
7	Galactose fermentation	→ 8	→ 9
8	Galactose growth	*Trichosporonoides oedocephalis*: p. 875	*Moniliella pollinis*: p. 787
9	Lactose growth	*Moniliella acetoabutens*: p. 785	→ 10
10	D-Xylose growth	*Trichosporonoides madida*: p. 873	*Trichosporonoides megachiliensis*: p. 874
11	Succinate growth	*Moniliella mellis*: p. 786	*Trichosporonoides nigrescens*: p. 875
12	Sucrose growth	→ 13	*Sporopachydermia quercuum*: p. 397
13	Lactose growth	→ 14	→ 16
14	Nitrate growth	→ 15	*Cystofilobasidium lari-marini*: p. 652
15	D-Glucosamine growth	*Mrakia frigida*: p. 676	*Cryptococcus aquaticus*: p. 750
16	Nitrate growth	*Mrakia frigida*: p. 676	→ 17
17	Galactose growth	*Filobasidium capsuligenum*: p. 664	*Xanthophyllomyces dendrorhous*: p. 718
18	Nitrate growth	→ 19	→ 151
19	Sucrose growth	→ 20	→ 131
20	Succinate growth	→ 21	→ 122
21	D-Glucosamine growth	→ 22	→ 48
22	Erythritol growth	→ 23	→ 37
23	Lactose growth	→ 24	→ 34
24	D-Xylose growth	→ 25	*Rhodotorula ferulica*: p. 810
25	Galactose growth	→ 26	→ 33
26	Growth at 37°C	→ 27	→ 28
27	Ethanol growth	*Pseudozyma antarctica*: p. 791	*Pseudozyma aphidis*: p. 792

#	Test	Positive	Negative
28	Ribitol growth	→ 29	→ 32
29	Inositol growth	→ 30	*Rhodotorula acheniorum*: p. 805
30	Galactitol growth	*Fibulobasidium inconspicuum*: p. 710	→ 31
31	Citrate growth	*Pseudozyma antarctica*: p. 791	*Pseudozyma prolifica*: p. 794
32	Citrate growth	*Pseudozyma tsukubaënsis*: p. 795	*Sympodiomycopsis paphiopedili*: p. 846
33	Growth at 37°C	*Ustilago maydis*: p. 796	*Fibulobasidium inconspicuum*: p. 710
34	Ethanol growth	→ 35	*Pseudozyma flocculosa*: p. 792
35	Inositol growth	→ 36	*Rhodotorula diffluens*: p. 810
36	Galactose growth	*Pseudozyma rugulosa*: p. 794	*Pseudozyma fusiformata*: p. 793
37	Inositol growth	→ 38	→ 43
38	Ribitol growth	→ 39	→ 42
39	Galactitol growth	→ 40	→ 41
40	L-Sorbose growth	*Cryptococcus aerius*: p. 747	*Filobasidium floriforme*: p. 666
41	α-Methyl-D-glucoside growth	*Filobasidium floriforme*: p. 666	*Itersonilia perplexans*: p. 775
42	Galactitol growth	*Cryptococcus yarrowii*: p. 765	*Itersonilia perplexans*: p. 775
43	Galactitol growth	*Rhodotorula muscorum*: p. 822	→ 44
44	α-Methyl-D-glucoside growth	→ 45	→ 46
45	Ethanol growth	*Rhodotorula fragaria*: p. 811	*Rhodotorula ingeniosa*: p. 817
46	Galactose growth	→ 47	*Leucosporidium fellii*: p. 671
47	L-Sorbose growth	*Erythrobasidium hasegawianum*: p. 654	*Itersonilia perplexans*: p. 775
48	Inositol growth	→ 49	→ 73
49	D-Xylose growth	→ 50	→ 69
50	Erythritol growth	→ 51	→ 63
51	Citrate growth	→ 52	→ 62
52	Ribitol growth	→ 53	→ 61
53	DL-Lactate growth	→ 54	→ 58
54	Galactose growth	→ 55	→ 56
55	Vitamin-free growth	*Tilletiopsis albescens*: p. 848 *Tilletiopsis pallescens*: p. 851	*Cryptococcus albidus*: p. 748
56	Vitamin-free growth	→ 57	*Cryptococcus albidus*: p. 748
57	Soluble starch growth	*Tilletiopsis pallescens*: p. 851	*Pseudozyma fusiformata*: p. 793
58	Vitamin-free growth	→ 59	→ 60
59	L-Arabinitol growth	*Tilletiopsis pallescens*: p. 851	*Trichosporon pullulans*: p. 869
60	10% NaCl/5% glucose growth	*Trichosporon pullulans*: p. 869	*Cryptococcus albidus*: p. 748
61	Gelatin liquefaction	*Cryptococcus macerans*: p. 760	*Cryptococcus albidus*: p. 748
62	Ribitol growth	*Tilletiopsis pallescens*: p. 851	*Sympodiomycopsis paphiopedili*: p. 846
63	Ethanol growth	→ 64	*Cryptococcus antarcticus*: p. 749
64	Ribitol growth	→ 65	→ 68
65	Galactitol growth	→ 66	→ 67
66	Starch formation	*Cryptococcus albidus*: p. 748	*Bullera pyricola*: p. 737
67	Maltose growth	*Cryptococcus albidus*: p. 748	*Cryptococcus kuetzingii*: p. 759
68	Raffinose growth	*Cryptococcus albidus*: p. 748	*Cryptococcus albidosimilis*: p. 747
69	Ribitol growth	→ 70	→ 72
70	Erythritol growth	*Tilletiopsis pallescens*: p. 851	→ 71
71	Raffinose growth	*Bullera punicea*: p. 739	*Sporobolomyces inositophilus*: p. 833
72	Citrate growth	*Rhodotorula hinnulea*: p. 816	*Rhodotorula phylloplana*: p. 824
73	Lactose growth	→ 74	→ 84
74	Raffinose growth	→ 75	→ 81
75	Maltose growth	→ 76	→ 80

#	Test	Positive	Negative
76	Soluble starch growth	→ 77	*Rhodotorula lactosa*: p. 818
77	L-Arabinitol growth	→ 78	*Sporobolomyces xanthus*: p. 841
78	Vitamin-free growth	*Tilletiopsis pallescens*: p. 851	→ 79
79	Pellicle formation	*Tilletiopsis fulvescens*: p. 850	*Tilletiopsis minor*: p. 851
80	Erythritol growth	*Bensingtonia ingoldii*: p. 724	*Bensingtonia miscanthi*: p. 726
81	Galactose growth	*Rhodotorula hordea*: p. 816	→ 82
82	D-Xylose growth	→ 83	*Bensingtonia phyllada*: p. 727
83	Soluble starch growth	*Sporobolomyces lactophilus*: p. 834	*Sporobolomyces tsugae*: p. 841
84	Erythritol growth	→ 85	→ 87
85	Pellicle formation	→ 86	*Rhodotorula bacarum*: p. 808
86	L-Sorbose growth	*Tilletiopsis pallescens*: p. 851	*Tilletiopsis washingtonensis*: p. 852
87	Starch formation	→ 88	→ 92
88	Ribitol growth	→ 89	→ 90
89	Galactose growth	*Bullera megalospora*: p. 735	*Bullera punicea*: p. 739
90	Galactose growth	*Cryptococcus bhutanensis*: p. 751	→ 91
91	*N*-Acetyl-D-glucosamine growth	*Cryptococcus friedmannii*: p. 754	*Cryptococcus vishniacii*: p. 765
92	Raffinose growth	→ 93	→ 115
93	Soluble starch growth	→ 94	→ 102
94	Ethanol growth	→ 95	→ 100
95	DL-Lactate growth	→ 96	→ 98
96	Citrate growth	→ 97	*Sporidiobolus salmonicolor*: p. 697 *Sporobolomyces roseus*: p. 836
97	2-Keto-D-gluconate growth	*Rhodosporidium babjevae*: p. 679	*Sporobolomyces roseus*: p. 836
98	Pellicle formation	*Sporobolomyces roseus*: p. 836	→ 99
99	α-Methyl-D-glucoside growth	*Rhodosporidium diobovatum*: p. 682	*Bullera megalospora*: p. 735
100	Pellicle formation	→ 101	*Bensingtonia naganoensis*: p. 726
101	Melibiose growth	*Tilletiopsis flava*: p. 849	*Sporobolomyces roseus*: p. 836
102	D-Xylose growth	→ 103	→ 114
103	Vitamin-free growth	→ 104	→ 111
104	Pellicle formation	→ 105	→ 106
105	Galactitol growth	*Sporidiobolus ruineniae*: p. 696 *Sporobolomyces roseus*: p. 836	*Sporidiobolus salmonicolor*: p. 697 *Sporobolomyces roseus*: p. 836
106	10% NaCl/5% glucose growth	→ 107	*Rhodosporidium paludigenum*: p. 687
107	Growth at 37°C	→ 108	→ 109
108	Inulin growth	*Rhodosporidium toruloides*: p. 690	*Rhodotorula glutinis*: p. 814
109	DL-Lactate growth	*Rhodosporidium fluviale*: p. 683	→ 110
110	Inulin growth	*Rhodosporidium kratochvilovae*: p. 684	*Rhodotorula graminis*: p. 815
111	Maltose growth	→ 112	*Sporobolomyces roseus*: p. 836 *Sporobolomyces salicinus*: p. 838
112	Pellicle formation	*Sporobolomyces roseus*: p. 836	→ 113
113	10% NaCl/5% glucose growth	*Rhodotorula glutinis*: p. 814	*Rhodosporidium malvinellum*: p. 686
114	Growth at 37°C	*Rhodotorula glutinis*: p. 814	*Sporobolomyces roseus*: p. 836 *Sporobolomyces subbrunneus*: p. 840
115	Maltose growth	→ 116	→ 121
116	Vitamin-free growth	→ 117	→ 119
117	10% NaCl/5% glucose growth	→ 118	*Sporidiobolus salmonicolor*: p. 697
118	Pellicle formation	*Sporidiobolus johnsonii*: p. 694	*Rhodotorula glutinis*: p. 814
119	Soluble starch growth	*Sporobolomyces griseoflavus*: p. 832	→ 120
120	10% NaCl/5% glucose growth	*Rhodotorula glutinis*: p. 814	*Rhodotorula aurantiaca*: p. 807

#	Test	Positive	Negative
121	Ethanol growth	*Sporidiobolus salmonicolor*: p. 697	*Sporobolomyces foliicola*: p. 831
122	Inositol growth	→ 123	→ 125
123	Melibiose growth	*Cystofilobasidium bisporidii*: p. 646	→ 124
124	Arbutin growth	*Cystofilobasidium infirmominiatum*: p. 650	*Cystofilobasidium capitatum*: p. 648
125	D-Glucosamine growth	*Leucosporidium scottii*: p. 673	→ 126
126	Ribitol growth	→ 127	→ 129
127	Galactitol growth	*Sporidiobolus ruineniae*: p. 696	→ 128
128	DL-Lactate growth	*Sporidiobolus salmonicolor*: p. 697	*Rhodosporidium sphaerocarpum*: p. 688
129	Vitamin-free growth	*Leucosporidium antarcticum*: p. 671	→ 130
130	Ethanol growth	*Kurtzmanomyces tardus*: p. 781	*Rhodotorula pilati*: p. 824
131	Inositol growth	→ 132	→ 134
132	L-Sorbose growth	→ 133	*Cryptococcus fuscescens*: p. 754
133	Lactose growth	*Cryptococcus terreus*: p. 764	*Cryptococcus feraegula*: p. 753
134	D-Glucosamine growth	→ 135	→ 139
135	Galactitol growth	*Cryptococcus huempii*: p. 757	→ 136
136	L-Sorbose growth	→ 137	→ 138
137	Maltose growth	*Rhodotorula buffonii*: p. 809	*Rhodotorula pustula*: p. 825
138	Galactose growth	*Sporobolomyces falcatus*: p. 831	*Rhodotorula foliorum*: p. 811
139	Erythritol growth	*Sterigmatomyces halophilus*: p. 845	→ 140
140	Succinate growth	→ 141	→ 150
141	Melibiose growth	*Rhodotorula javanica*: p. 818	→ 142
142	Raffinose growth	→ 143	→ 144
143	Starch formation	*Cryptococcus vishniacii*: p. 765	*Sporobolomyces roseus*: p. 836
144	Ethanol growth	→ 145	→ 147
145	Galactitol growth	→ 146	*Bensingtonia ciliata*: p. 724
146	L-Sorbose growth	*Rhodosporidium lusitaniae*: p. 685	*Sporobolomyces kluyveri-nielii*: p. 834
147	Ribitol growth	→ 148	→ 149
148	L-Sorbose growth	*Bensingtonia yuccicola*: p. 729	*Kurtzmanomyces nectairei*: p. 780
149	Galactose growth	*Rhodotorula sonckii*: p. 826	*Cryptococcus vishniacii*: p. 765
150	Ribitol growth	*Rhodotorula araucariae*: p. 806	*Leucosporidium antarcticum*: p. 671
151	Inositol growth	→ 152	→ 239
152	Sucrose growth	→ 153	→ 229
153	Maltose growth	→ 154	→ 225
154	Vitamin-free growth	→ 155	→ 160
155	Ribitol growth	→ 156	*Cryptococcus podzolicus*: p. 762
156	Galactitol growth	→ 157	→ 159
157	Melibiose growth	*Cryptococcus laurentii*: p. 759	→ 158
158	Erythritol growth	*Sirobasidium intermedium*: p. 710	*Holtermannia corniformis*: p. 711
159	Melibiose growth	*Hyalodendron lignicola*: p. 773	*Cryptococcus curvatus*: p. 752
160	α-Methyl-D-glucoside growth	→ 161	→ 214
161	Ethanol growth	→ 162	→ 199
162	Erythritol growth	→ 163	→ 186
163	Lactose growth	→ 164	→ 184
164	Melibiose growth	→ 165	→ 174
165	Growth at 37°C	*Trichosporon mucoides*: p. 868	→ 166
166	Galactitol growth	→ 167	→ 172
167	D-Glucosamine growth	→ 168	→ 171
168	DL-Lactate growth	→ 169	*Sterigmatosporidium polymorphum*: p. 700

#	Test	Positive	Negative
169	Inulin growth	*Bulleromyces albus*: p. 641 *Cryptococcus humicolus*: p. 757	→ 170
170	50% Glucose growth	*Trichosporon jirovecii*: p. 864	*Cryptococcus humicolus*: p. 757
171	Inulin growth	*Bulleromyces albus*: p. 641	*Cryptococcus laurentii*: p. 759
172	L-Arabinitol growth	→ 173	*Bullera miyagiana*: p. 736
173	Inulin growth	*Bulleromyces albus*: p. 641	*Trichosporon cutaneum*: p. 860 *Trichosporon moniliiforme*: p. 866
174	50% Glucose growth	→ 175	→ 181
175	L-Arabinitol growth	→ 176	→ 180
176	*N*-Acetyl-D-glucosamine growth	→ 177	*Trichosporon faecale*: p. 862
177	D-Glucosamine growth	→ 178	→ 179
178	Growth at 40°C	*Trichosporon asahii*: p. 857	*Trichosporon asteroides*: p. 858
179	Nitrite growth	*Trichosporon coremiiforme*: p. 859	*Trichosporon asteroides*: p. 858
180	L-Rhamnose growth	*Trichosporon ovoides*: p. 869	*Trichosporon inkin*: p. 863
181	Inulin growth	*Bulleromyces albus*: p. 641	→ 182
182	Galactitol growth	→ 183	*Cryptococcus curvatus*: p. 752
183	D-Glucosamine growth	*Fibulobasidium inconspicuum*: p. 710	*Cryptococcus heveanensis*: p. 756
184	50% Glucose growth	*Cryptococcus amylolentus*: p. 749	→ 185
185	Pellicle formation	*Bullera sinensis*: p. 739	*Cryptococcus luteolus*: p. 760
186	L-Rhamnose growth	→ 187	*Trichosporon montevideense*: p. 867
187	Lactose growth	→ 188	→ 197
188	Inulin growth	*Bulleromyces albus*: p. 641	→ 189
189	D-Glucosamine growth	→ 190	→ 196
190	Galactitol growth	→ 191	→ 194
191	L-Sorbose growth	→ 192	→ 193
192	Melibiose growth	*Tremella encephala*: p. 714 *Tremella indecorata*: p. 716 *Trichosporon laibachii*: p. 865	*Tremella aurantia*: p. 712 *Tremella encephala*: p. 714 *Tremella indecorata*: p. 716
193	Melibiose growth	*Bullera pseudoalba*: p. 738	*Tremella aurantia*: p. 712
194	Ribitol growth	→ 195	*Trichosporon sporotrichoides*: p. 870
195	Melibiose growth	*Trichosporon loubieri*: p. 866	*Tremella aurantia*: p. 712
196	Melibiose growth	*Cryptococcus laurentii*: p. 759	*Bullera dendrophila*: p. 733
197	L-Sorbose growth	→ 198	*Filobasidiella neoformans* var. *neoformans*: p. 656 *Filobasidiella neoformans* var. *bacillispora*: p. 657
198	D-Glucosamine growth	*Tremella indecorata*: p. 716	*Holtermannia corniformis*: p. 711
199	Inulin growth	→ 200	→ 202
200	Lactose growth	→ 201	*Filobasidium uniguttulatum*: p. 667
201	Starch formation	*Bulleromyces albus*: p. 641	*Cryptococcus flavus*: p. 754
202	D-Glucosamine growth	→ 203	→ 212
203	Melibiose growth	→ 204	→ 211
204	Soluble starch growth	→ 205	*Cryptococcus luteolus*: p. 760
205	Galactitol growth	→ 206	→ 210
206	Starch formation	→ 207	*Fellomyces polyborus*: p. 771 *Trimorphomyces papilionaceus*: p. 717
207	L-Sorbose growth	→ 208	→ 209
208	Pellicle formation	*Bullera variabilis*: p. 740 *Fellomyces penicillatus*: p. 770	*Fellomyces penicillatus*: p. 770 *Trimorphomyces papilionaceus*: p. 717
209	Nitrite growth	*Bullera oryzae*: p. 736	*Bullera variabilis*: p. 740
210	DL-Lactate growth	*Trimorphomyces papilionaceus*: p. 717	*Kockovaella thailandica*: p. 778
211	Ribitol growth	*Tremella moriformis*: p. 717	*Cryptococcus ater*: p. 750

#	Test	Positive	Negative
212	*N*-Acetyl-D-glucosamine growth	*Cryptococcus hungaricus*: p. 758	→ 213
213	Ribitol growth	*Cryptococcus laurentii*: p. 759	*Cryptococcus magnus*: p. 761
214	Erythritol growth	→ 215	→ 216
215	Galactitol growth	*Fellomyces fuzhouensis*: p. 769	*Cryptococcus curvatus*: p. 752
216	DL-Lactate growth	→ 217	→ 223
217	Raffinose growth	→ 218	→ 221
218	Galactose growth	→ 219	*Trichosporon dulcitum*: p. 861
219	D-Glucosamine growth	*Bullera armeniaca*: p. 732	→ 220
220	L-Sorbose growth	*Cryptococcus hungaricus*: p. 758	*Bullera globispora*: p. 734
221	Galactitol growth	*Holtermannia corniformis*: p. 711	→ 222
222	Galactose growth	*Trichosporon brassicae*: p. 859	*Trichosporon gracile*: p. 862
223	Galactitol growth	*Kockovaella imperatae*: p. 777	→ 224
224	L-Sorbose growth	*Kockovaella thailandica*: p. 778	*Itersonilia perplexans*: p. 775
225	Erythritol growth	→ 226	→ 227
226	Galactitol growth	*Fellomyces fuzhouensis*: p. 769	*Tsuchiyaea wingfieldii*: p. 878
227	Galactitol growth	*Cryptococcus dimennae*: p. 753	→ 228
228	Galactose growth	*Itersonilia perplexans*: p. 775	*Trichosporon gracile*: p. 862
229	Succinate growth	→ 230	→ 238
230	Citrate growth	→ 231	→ 236
231	L-Rhamnose growth	→ 232	*Trichosporon gracile*: p. 862
232	Lactose growth	→ 233	→ 234
233	Galactitol growth	*Sirobasidium magnum*: p. 711	*Trichosporon asahii*: p. 857
234	Maltose growth	→ 235	*Cryptococcus skinneri*: p. 763
235	Soluble starch growth	*Holtermannia corniformis*: p. 711	*Tremella foliacea*: p. 714
236	Ribitol growth	*Sporopachydermia lactativora*: p. 396	→ 237
237	D-Xylose growth	*Cryptococcus gastricus*: p. 755	*Cryptococcus gilvescens*: p. 756
238	Ethanol growth	*Cryptococcus marinus*: p. 762	*Filobasidium globisporum*: p. 667
239	Melibiose growth	→ 240	→ 247
240	L-Sorbose growth	→ 241	→ 243
241	Ethanol growth	→ 242	→ 210
242	Erythritol growth	*Tremella fuciformis*: p. 715	*Ascoidea africana*: p. 137
243	Galactitol growth	*Bullera crocea*: p. 733	→ 244
244	Erythritol growth	*Hyalodendron lignicola*: p. 773	→ 245
245	α-Methyl-D-glucoside growth	*Sporobolomyces ruber*: p. 837	→ 246
246	Galactose growth	*Itersonilia perplexans*: p. 775	*Tilletiaria anomala*: p. 703
247	Erythritol growth	→ 248	→ 260
248	Maltose growth	→ 249	→ 255
249	L-Rhamnose growth	*Trichosporon asahii*: p. 857	→ 250
250	Sucrose growth	→ 251	→ 253
251	Ribitol growth	→ 252	*Trichosporon aquatile*: p. 856
252	Lactose growth	*Tremella cinnabarina*: p. 713	*Tremella globispora*: p. 715
253	D-Xylose growth	*Tremella brasiliensis*: p. 712	→ 254
254	Galactose growth	*Prototheca stagnora*: p. 885	*Prototheca moriformis*: p. 884
255	Sucrose growth	→ 256	→ 258
256	Galactitol growth	*Sterigmatomyces elviae*: p. 844	→ 257
257	α-Methyl-D-glucoside growth	*Agaricostilbum hyphaenes*: p. 639	*Rhodotorula acuta*: p. 805
258	D-Xylose growth	→ 259	*Prototheca ulmea*: p. 885
259	2-Keto-D-gluconate growth	*Sterigmatomyces halophilus*: p. 845	*Tremella mesenterica*: p. 716

#	Test	Positive	Negative
260	Starch formation	→ 261	→ 265
261	Ribitol growth	*Tremella coalescens*: p. 713	→ 262
262	Maltose growth	→ 263	→ 264
263	Melezitose growth	*Cryptococcus vishniacii*: p. 765	*Filobasidium elegans*: p. 665
264	Sucrose growth	*Cryptococcus consortionis*: p. 751	*Chionosphaera apobasidialis*: p. 644
265	Inulin growth	→ 266	→ 270
266	Sucrose growth	→ 267	→ 268
267	L-Arabinitol growth	*Sporobolomyces alborubescens*: p. 830	*Itersonilia perplexans*: p. 775
268	Galactose growth	*Prototheca stagnora*: p. 885	→ 269
269	Maltose growth	*Prototheca moriformis*: p. 884	*Prototheca ulmea*: p. 885
270	α-Methyl-D-glucoside growth	→ 271	→ 275
271	Sucrose growth	→ 272	→ 268
272	2-Keto-D-gluconate growth	*Sporobolomyces oryzicola*: p. 835	→ 273
273	Pellicle formation	*Sporidiobolus pararoseus*: p. 694	→ 274
274	Soluble starch growth	*Rhodotorula marina*: p. 819	*Rhodotorula mucilaginosa*: p. 820
275	Soluble starch growth	→ 276	→ 280
276	Melezitose growth	→ 277	→ 279
277	L-Sorbose growth	*Rhodotorula bogoriensis*: p. 808	→ 278
278	Galactose growth	*Itersonilia perplexans*: p. 775	*Bensingtonia yamatoana*: p. 729
279	Ethanol growth	*Bensingtonia subrosea*: p. 728	*Sporobolomyces sasicola*: p. 839
280	Succinate growth	→ 281	→ 312
281	50% Glucose growth	→ 282	→ 286
282	Galactose growth	→ 283	→ 285
283	D-Xylose growth	*Rhodotorula nothofagi*: p. 823	→ 284
284	Maltose growth	*Prototheca stagnora*: p. 885	*Prototheca wickerhamii*: p. 886
285	D-Xylose growth	*Rhodotorula lignophila*: p. 818	→ 269
286	Raffinose growth	*Rhodotorula mucilaginosa*: p. 820	→ 287
287	D-Glucosamine growth	→ 288	→ 291
288	Galactose growth	→ 284	→ 289
289	Maltose growth	*Prototheca moriformis*: p. 884	→ 290
290	Vitamin-free growth	*Rhodotorula philyla*: p. 823	*Prototheca ulmea*: p. 885
291	D-Xylose growth	→ 292	→ 301
292	Sucrose growth	→ 293	→ 298
293	Galactitol growth	*Sporobolomyces phyllomatis*: p. 835	→ 294
294	L-Rhamnose growth	*Sporobolomyces elongatus*: p. 830	→ 295
295	Maltose growth	→ 296	→ 297
296	L-Sorbose growth	*Bensingtonia intermedia*: p. 725	*Bensingtonia yamatoana*: p. 729
297	Citrate growth	*Bensingtonia intermedia*: p. 725	*Rhodotorula minuta*: p. 820
298	Ethanol growth	→ 299	→ 300
299	Galactitol growth	*Rhodotorula futronensis*: p. 813	*Bensingtonia intermedia*: p. 725
300	Galactose growth	*Rhodotorula armeniaca*: p. 806	*Sporobolomyces gracilis*: p. 832
301	DL-Lactate growth	→ 302	→ 309
302	Galactose growth	→ 303	→ 306
303	Maltose growth	→ 304	→ 305
304	Trehalose growth	*Rhodosporidium dacryoideum*: p. 680	*Prototheca stagnora*: p. 885
305	D-Gluconate growth	*Rhodosporidium dacryoideum*: p. 680	*Prototheca wickerhamii*: p. 886
306	Trehalose growth	*Sporobolomyces singularis*: p. 839	→ 307
307	Maltose growth	*Prototheca moriformis*: p. 884	→ 308
308	D-Mannitol growth	*Rhodotorula hylophila*: p. 817	*Prototheca ulmea*: p. 885

#	Test	Positive	Negative
309	Ribitol growth	→ 310	→ 311
310	D-Mannitol growth	*Bensingtonia intermedia*: p. 725	→ 268
311	D-Mannitol growth	*Rhodotorula auriculariae*: p. 807	→ 268
312	D-Mannitol growth	→ 313	→ 315
313	Ethanol growth	→ 314	*Reniforma strues*: p. 798
314	Galactitol growth	*Rhodotorula fujisanensis*: p. 812	*Bensingtonia yamatoana*: p. 729
315	Maltose growth	→ 316	→ 317
316	Hexadecane growth	*Prototheca zopfii* var. *hydrocarbonea*: p. 886	*Prototheca moriformis*: p. 884 *Prototheca zopfii* var. *zopfii*: p. 886 *Prototheca zopfii* var. *portoricensis*: p. 886
317	Galactose growth	*Prototheca wickerhamii*: p. 886	*Prototheca ulmea*: p. 885
318	Inositol growth	→ 319	→ 361
319	Nitrate growth	→ 320	→ 328
320	Glucose fermentation	→ 321	→ 324
321	Galactose fermentation	→ 322	→ 323
322	Galactitol growth	*Pichia ofunaensis*: p. 328	*Arxula adeninivorans*: p. 441
323	Erythritol growth	*Candida incommunis*: p. 513	*Candida valdiviana*: p. 566
324	Melibiose growth	→ 325	*Arxula terrestris*: p. 442
325	10% NaCl/5% glucose growth	→ 326	→ 327
326	Inulin growth	*Aureobasidium pullulans*: p. 123	*Stephanoascus smithiae*: p. 402
327	Galactitol growth	*Stephanoascus smithiae*: p. 402	*Candida bertae*: p. 486
328	D-Glucosamine growth	→ 329	→ 341
329	Ribitol growth	→ 330	*Candida paludigena*: p. 536
330	L-Sorbose growth	→ 331	→ 340
331	Erythritol growth	→ 332	→ 339
332	Ethanol growth	→ 333	→ 338
333	Melibiose growth	→ 334	→ 336
334	Galactitol growth	→ 335	*Candida santjacobensis*: p. 550
335	Raffinose growth	*Blastobotrys proliferans*: p. 447	*Blastobotrys nivea*: p. 446
336	L-Rhamnose growth	→ 337	*Candida auringiensis*: p. 484
337	Melezitose growth	*Candida blankii*: p. 487	*Blastobotrys capitulata*: p. 445
338	Glucose fermentation	*Blastobotrys aristata*: p. 444	*Candida chiropterorum*: p. 495
339	Glucose fermentation	*Zygoascus hellenicus*: p. 422	*Ascoidea hylecoeti*: p. 138
340	Ethanol growth	*Pichia tannicola*: p. 345	*Sympodiomyces parvus*: p. 603
341	Sucrose growth	→ 342	→ 357
342	DL-Lactate growth	→ 343	→ 349
343	L-Rhamnose growth	→ 344	→ 346
344	Lactose growth	→ 345	*Stephanoascus ciferrii*: p. 400
345	Melibiose growth	*Myxozyma monticola*: p. 595	*Candida blankii*: p. 487
346	Galactitol growth	*Myxozyma geophila*: p. 593	→ 347
347	Glucose fermentation	*Saccharomycopsis fibuligera*: p. 377	→ 348
348	Ribitol growth	*Myxozyma udenii*: p. 596	*Candida castrensis*: p. 493
349	Melibiose growth	→ 350	→ 355
350	Erythritol growth	→ 351	→ 353
351	L-Sorbose growth	*Lipomyces starkeyi*: p. 252 *Lipomyces tetrasporus*: p. 252	→ 352
352	0.01% Cycloheximide growth	*Lipomyces starkeyi*: p. 252	*Ascoidea rubescens*: p. 139
353	Growth at 37°C	*Dipodascopsis uninucleata* var. *uninucleata*: p. 179	→ 354
354	Vitamin-free growth	*Lipomyces starkeyi*: p. 252	*Myxozyma kluyveri*: p. 593

#	Test	Positive	Negative
355	Erythritol growth	*Candida blankii*: p. 487	→ 356
356	Lactose growth	*Dipodascopsis tothii*: p. 178	*Pichia inositovora*: p. 313
357	Erythritol growth	→ 358	→ 359
358	L-Sorbose growth	*Sporopachydermia cereana*: p. 395	*Saccharomycopsis synnaedendra*: p. 382
359	Ribitol growth	→ 360	*Zygozyma oligophaga*: p. 434
360	Galactose growth	*Myxozyma melibiosi*: p. 594	*Dipodascopsis uninucleata* var. *wickerhamii*: p. 179
361	Nitrate growth	→ 362	→ 442
362	Vitamin-free growth	→ 363	→ 376
363	Erythritol growth	→ 364	→ 367
364	L-Rhamnose growth	→ 365	→ 366
365	Melibiose growth	*Pichia sydowiorum*: p. 344	*Pichia ciferrii*: p. 299
366	Inulin growth	*Pichia lynferdii*: p. 317	*Pichia anomala*: p. 287
367	Raffinose growth	→ 368	→ 372
368	L-Sorbose growth	→ 369	→ 371
369	Glucose fermentation	*Citeromyces matritensis*: p. 146	→ 370
370	Melibiose growth	*Saitoella complicata*: p. 600	
371	Maltose growth	*Pichia jadinii*: p. 314 *Williopsis saturnus* var. *saturnus*: p. 416	*Williopsis saturnus* var. *saturnus*: p. 416 *Williopsis saturnus* var. *suaveolens*: p. 417
372	Glucose fermentation	→ 373	→ 375
373	Sucrose growth	*Candida vartiovaarae*: p. 567	→ 374
374	D-Xylose growth	*Williopsis saturnus* var. *mrakii*: p. 417	*Candida berthetii*: p. 487
375	D-Mannitol growth	*Pichia dryadoides*: p. 301	*Ascoidea corymbosa*: p. 137
376	Inulin growth	→ 377	→ 383
377	Ethanol growth	*Protomyces lactucaedebilis*: p. 355	→ 378
378	Ribitol growth	→ 379	→ 381
379	Gelatin liquefaction	*Protomyces inundatus*: p. 354 *Protomyces pachydermus*: p. 356	→ 380
380	Cellobiose growth	*Protomyces inouyei*: p. 354	*Protomyces inundatus*: p. 354
381	Gelatin liquefaction	*Protomyces pachydermus*: p. 356	→ 382
382	Cellobiose growth	*Protomyces gravidus*: p. 354	*Protomyces macrosporus*: p. 355
383	Starch formation	→ 384	→ 385
384	Glucose fermentation	*Candida glucosophila*: p. 510	*Oosporidium margaritiferum*: p. 598
385	Erythritol growth	→ 386	→ 401
386	Glucose fermentation	→ 387	→ 398
387	Raffinose growth	*Pichia subpelliculosa*: p. 344	→ 388
388	Sucrose growth	→ 389	→ 394
389	Lactose growth	*Candida chilensis*: p. 495	→ 390
390	D-Glucosamine growth	→ 391	→ 393
391	L-Sorbose growth	→ 392	*Ambrosiozyma platypodis*: p. 132
392	DL-Lactate growth	*Candida ishiwadae*: p. 516	*Pichia holstii*: p. 312
393	Galactose fermentation	*Pichia silvicola*: p. 340	*Pichia angusta*: p. 286
394	Cellobiose growth	→ 395	→ 397
395	D-Glucosamine growth	*Pichia capsulata*: p. 296	→ 396
396	Galactose fermentation	*Pichia silvicola*: p. 340	*Pichia glucozyma*: p. 307
397	Galactose fermentation	*Candida nanaspora*: p. 530	*Candida boidinii*: p. 488
398	Citrate growth	→ 399	→ 400
399	Sucrose growth	*Pichia methylivora*: p. 321	*Pichia philodendri*: p. 333
400	L-Sorbose growth	*Pichia finlandica*: p. 305	*Pichia henricii*: p. 311
401	Soluble starch growth	→ 402	→ 405

#	Test	Positive	Negative
402	Ribitol growth	→ 403	→ 404
403	Galactose growth	*Pichia holstii*: p. 312	*Candida ernobii*: p. 501
404	D-Mannitol growth	*Pichia fabianii*: p. 303	*Ascoidea corymbosa*: p. 137
405	Melezitose growth	→ 406	→ 416
406	L-Rhamnose growth	→ 407	→ 414
407	Raffinose growth	*Pichia petersonii*: p. 332	→ 408
408	D-Glucosamine growth	*Candida populi*: p. 541	→ 409
409	Galactose fermentation	*Pichia silvicola*: p. 340	→ 410
410	Growth at 37°C	→ 411	→ 412
411	Glucose fermentation	*Pichia bimundalis*: p. 291	*Pichia canadensis*: p. 295
412	Glucose fermentation	*Pichia americana*: p. 283 *Pichia bispora*: p. 291	→ 413
413	Pellicle formation	*Pichia alni*: p. 282	*Pichia bispora*: p. 291
414	D-Mannitol growth	→ 415	*Dekkera anomala*: p. 174 *Dekkera bruxellensis*: p. 175
415	Galactose growth	*Williopsis pratensis*: p. 415	*Pichia euphorbiiphila*: p. 302
416	Galactitol growth	*Candida vanderwaltii*: p. 566	→ 417
417	Glucose fermentation	→ 418	→ 440
418	D-Mannitol growth	→ 419	→ 436
419	*N*-Acetyl-D-glucosamine growth	→ 420	→ 423
420	L-Sorbose growth	*Candida methanosorbosa*: p. 526	→ 421
421	Cellobiose growth	→ 422	*Candida nitratophila*: p. 531
422	Galactose fermentation	*Candida versatilis*: p. 567	*Candida wickerhamii*: p. 570
423	Hexadecane growth	*Candida vaccinii*: p. 565	→ 424
424	DL-Lactate growth	→ 425	→ 429
425	Raffinose growth	*Williopsis saturnus* var. *subsufficiens*: p. 417	→ 426
426	L-Sorbose growth	→ 427	→ 428
427	Ribitol growth	*Pichia silvicola*: p. 340	*Williopsis californica*: p. 413
428	Ribitol growth	*Pichia silvicola*: p. 340	*Candida norvegica*: p. 532
429	Galactose growth	→ 430	→ 434
430	D-Gluconate growth	→ 431	→ 433
431	Galactose fermentation	*Pichia silvicola*: p. 340	→ 432
432	Growth at 37°C	*Candida magnoliae*: p. 521	*Candida etchellsii*: p. 502
433	Ribitol growth	*Pachysolen tannophilus*: p. 271	*Candida etchellsii*: p. 502
434	Ribitol growth	→ 435	*Candida etchellsii*: p. 502
435	Cellobiose growth	*Pichia minuta* var. *minuta*: p. 324	*Candida pignaliae*: p. 539
436	Ribitol growth	*Pichia silvicola*: p. 340	→ 437
437	D-Xylose growth	*Candida glucosophila*: p. 510	→ 438
438	50% Glucose growth	→ 439	*Dekkera anomala*: p. 174 *Dekkera bruxellensis*: p. 175
439	Sucrose growth	*Candida lactis-condensi*: p. 519	*Candida etchellsii*: p. 502
440	Ribitol growth	*Pichia minuta* var. *nonfermentans*: p. 324	→ 441
441	Cellobiose growth	*Pichia populi*: p. 335	*Candida etchellsii*: p. 502 *Wickerhamiella domercqiae*: p. 411
442	Erythritol growth	→ 443	→ 552
443	Sucrose growth	→ 444	→ 508
444	Galactose growth	→ 445	→ 504
445	Starch formation	→ 446	→ 448
446	Glucose fermentation	*Blastobotrys aristata*: p. 444	→ 447

#	Test	Positive	Negative
447	0.1% Cycloheximide growth	*Lipomyces starkeyi*: p. 252 *Lipomyces tetrasporus*: p. 252	*Lipomyces lipofer*: p. 251
448	D-Xylose growth	→ 449	→ 502
449	Vitamin-free growth	→ 450	→ 462
450	Pellicle formation	→ 451	→ 457
451	Galactitol growth	→ 452	→ 456
452	L-Sorbose growth	→ 453	*Pichia heimii*: p. 311
453	Melibiose growth	→ 454	→ 455
454	Raffinose fermentation	*Debaryomyces polymorphus*: p. 166	*Debaryomyces vanrijiae* var. *vanrijiae*: p. 170
455	Raffinose fermentation	*Debaryomyces polymorphus*: p. 166	*Debaryomyces vanrijiae* var. *yarrowii*: p. 170
456	L-Rhamnose growth	*Debaryomyces robertsiae*: p. 168	*Pichia burtonii*: p. 293
457	Growth at 37°C	→ 458	→ 459
458	Melibiose growth	*Candida silvicultrix*: p. 555	*Candida homilentoma*: p. 512
459	Citrate growth	→ 460	→ 461
460	Glucose fermentation	*Candida fennica*: p. 503	*Lipomyces starkeyi*: p. 252
461	Glucose fermentation	*Candida rhagii*: p. 545	*Lipomyces starkeyi*: p. 252
462	Ethanol growth	→ 463	→ 501
463	Raffinose growth	→ 464	→ 482
464	2-Keto-D-gluconate growth	→ 465	→ 480
465	D-Gluconate growth	→ 466	→ 478
466	10% NaCl/5% glucose growth	→ 467	→ 476
467	Hexadecane growth	→ 468	→ 473
468	Raffinose fermentation	*Candida membranifaciens*: p. 525 *Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	→ 469
469	L-Rhamnose growth	→ 470	→ 472
470	Galactose fermentation	→ 471	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162 *Debaryomyces udenii*: p. 169
471	Growth at 37°C	*Debaryomyces hansenii* var. *fabryi*: p. 162 *Pichia mexicana*: p. 322 *Pichia scolyti*: p. 339	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162 *Pichia scolyti*: p. 339
472	Galactitol growth	*Candida friedrichii*: p. 506 *Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	*Candida shehatae* var. *shehatae*: p. 552 *Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162
473	Growth at 37°C	→ 474	→ 475
474	Propane 1,2 diol growth	*Candida entomophila*: p. 500	*Debaryomyces hansenii* var. *fabryi*: p. 162
475	Galactose fermentation	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162 *Debaryomyces pseudopolymorphus*: p. 167	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162 *Debaryomyces maramus*: p. 163 *Debaryomyces nepalensis*: p. 164
476	Melibiose growth	*Pichia scolyti*: p. 339	→ 477
477	L-Rhamnose growth	*Candida tenuis*: p. 561	*Candida shehatae* var. *shehatae*: p. 552
478	Melibiose growth	*Candida entomophila*: p. 500	→ 479
479	DL-Lactate growth	*Candida shehatae* var. *lignosa*: p. 552	*Candida shehatae* var. *shehatae*: p. 552
480	L-Rhamnose growth	→ 481	*Candida entomophila*: p. 500
481	Raffinose fermentation	*Candida silvanorum*: p. 554	*Candida insectorum*: p. 515
482	L-Rhamnose growth	→ 483	→ 491
483	Galactitol growth	→ 484	→ 485
484	Galactose fermentation	*Candida dendronema*: p. 498	*Pichia triangularis*: p. 348
485	Citrate growth	→ 486	*Pichia naganishii*: p. 325
486	DL-Lactate growth	→ 487	→ 488

#	Test	Positive	Negative
487	Lactose growth	*Candida tenuis*: p. 561 *Pichia stipitis*: p. 342	*Pichia nakazawae* var. *nakazawae*: p. 327 *Pichia stipitis*: p. 342
488	Lactose growth	*Candida tenuis*: p. 561	→ 489
489	Galactose fermentation	→ 490	*Candida atlantica*: p. 483
490	L-Sorbose growth	*Candida naeodendra*: p. 529	*Candida homilentoma*: p. 512
491	Glucose fermentation	→ 492	*Candida aaseri*: p. 476
492	Maltose growth	→ 493	*Pichia methanolica*: p. 321
493	Growth at 37°C	→ 494	→ 496
494	2-Keto-D-gluconate growth	*Pichia philogaea*: p. 333	→ 495
495	Pellicle formation	*Candida diddensiae*: p. 498	*Candida butyri*: p. 491
496	DL-Lactate growth	*Candida shehatae* var. *lignosa*: p. 552	→ 497
497	Soluble starch growth	→ 498	→ 499
498	Galactose fermentation	*Candida shehatae* var. *shehatae*: p. 552	*Pichia nakazawae* var. *akitaensis*: p. 327
499	L-Arabinitol growth	*Candida atmosphaerica*: p. 483	→ 500
500	L-Arabinose growth	*Candida ergastensis*: p. 501	*Candida coipomoensis*: p. 496
501	Lactose growth	*Blastobotrys aristata*: p. 444	*Candida peltata*: p. 538
502	Glucose fermentation	*Candida sophiae-reginae*: p. 556	→ 503
503	Melibiose growth	*Lipomyces starkeyi*: p. 252	*Debaryomyces melissophilus*: p. 164
504	Growth at 37°C	*Ambrosiozyma cicatricosa*: p. 130 *Ambrosiozyma monospora*: p. 131	→ 505
505	Melibiose growth	*Lipomyces starkeyi*: p. 252	→ 506
506	L-Sorbose growth	*Candida mesenterica*: p. 525	→ 507
507	L-Rhamnose growth	*Ambrosiozyma philentoma*: p. 131	*Ambrosiozyma ambrosiae*: p. 129
508	Melibiose growth	→ 509	→ 511
509	Glucose fermentation	→ 510	*Pichia castillae*: p. 298
510	Cellobiose growth	*Stephanoascus farinosus*: p. 401	*Pichia tannicola*: p. 345
511	Glucose fermentation	→ 512	→ 543
512	Ethanol growth	→ 513	→ 542
513	L-Rhamnose growth	→ 514	→ 516
514	Galactose fermentation	*Candida succiphila*: p. 559	→ 515
515	Cellobiose growth	*Pichia pini*: p. 334	*Candida boidinii*: p. 488
516	2-Keto-D-gluconate growth	→ 517	→ 530
517	Maltose growth	→ 518	→ 521
518	Galactose growth	→ 519	→ 520
519	D-Gluconate growth	*Candida sequanensis*: p. 551	*Stephanoascus farinosus*: p. 401
520	Growth at 37°C	*Saccharomycopsis malanga*: p. 379	*Saccharomycopsis capsularis*: p. 375
521	Galactose fermentation	→ 522	→ 523
522	Cellobiose growth	*Stephanoascus farinosus*: p. 401	*Candida schatavii*: p. 551
523	Trehalose growth	→ 524	*Candida boidinii*: p. 488
524	Pellicle formation	→ 525	→ 526
525	True hyphae	*Stephanoascus farinosus*: p. 401	*Candida boleticola*: p. 489
526	Hexadecane growth	→ 527	→ 528
527	Cellobiose growth	*Stephanoascus farinosus*: p. 401	*Candida laureliae*: p. 519
528	Cellobiose growth	→ 529	*Candida cantarellii*: p. 492
529	Galactose growth	*Stephanoascus farinosus*: p. 401	*Pichia pini*: p. 334
530	Ribitol growth	→ 531	*Candida pini*: p. 540
531	Galactose growth	→ 532	→ 536
532	Growth at 37°C	→ 533	→ 534
533	Maltose fermentation	*Pichia acaciae*: p. 282	*Pichia farinosa*: p. 304

#	Test	Positive	Negative
534	True hyphae	*Stephanoascus farinosus*: p. 401	→ 535
535	Galactitol growth	*Pichia methanolica*: p. 321	*Candida conglobata*: p. 496
536	Trehalose growth	→ 537	→ 540
537	Cellobiose growth	→ 538	→ 539
538	DL-Lactate growth	*Candida ovalis*: p. 535	*Pichia pini*: p. 334
539	L-Sorbose growth	*Pichia trehalophila*: p. 347	*Candida llanquihuensis*: p. 520
540	Cellobiose growth	*Pichia kodamae*: p. 317	→ 541
541	DL-Lactate growth	*Candida boidinii*: p. 488	*Williopsis salicorniae*: p. 416
542	Galactose growth	*Blastobotrys arbuscula*: p. 444 *Stephanoascus farinosus*: p. 401	*Pichia pini*: p. 334
543	D-Mannitol growth	→ 544	→ 268
544	Maltose growth	→ 545	→ 547
545	Lactose growth	*Blastobotrys elegans*: p. 446	→ 546
546	L-Sorbose growth	*Pichia media*: p. 318	*Debaryomyces coudertii*: p. 160
547	Galactitol growth	→ 548	→ 550
548	Trehalose growth	*Candida nemodendra*: p. 531	→ 549
549	D-Xylose growth	*Pichia haplophila*: p. 310	*Candida fermenticarens*: p. 504
550	Trehalose growth	→ 551	*Yarrowia lipolytica*: p. 420
551	Galactose growth	*Candida psychrophila*: p. 542	*Pichia pini*: p. 334
552	True hyphae	→ 553	→ 609
553	L-Rhamnose growth	→ 554	→ 560
554	Melezitose growth	→ 555	*Pichia meyerae*: p. 323
555	Galactose growth	*Candida tenuis*: p. 561	→ 556
556	Glucose fermentation	→ 557	*Pichia canadensis*: p. 295
557	Ribitol growth	*Pichia wickerhamii*: p. 349	→ 558
558	L-Arabinose growth	→ 559	*Pichia rhodanensis*: p. 338
559	Sucrose fermentation	*Pichia euphorbiae*: p. 301	*Pichia mississippiensis*: p. 325
560	Cellobiose growth	→ 561	→ 580
561	D-Mannitol growth	→ 562	→ 578
562	Galactose growth	→ 563	→ 573
563	Trehalose growth	→ 564	→ 571
564	Growth at 37°C	*Candida tropicalis*: p. 563 *Candida viswanathii*: p. 570	→ 565
565	DL-Lactate growth	→ 566	→ 568
566	Ethanol growth	→ 567	*Protomyces inouyei*: p. 354
567	L-Sorbose growth	*Cephaloascus albidus*: p. 143	*Candida lyxosophila*: p. 521
568	Sucrose growth	→ 569	*Stephanoascus farinosus*: p. 401
569	Galactose fermentation	*Candida railenensis*: p. 544	→ 570
570	L-Sorbose growth	*Metschnikowia hawaiiensis*: p. 261	*Candida tanzawaensis*: p. 561
571	Glucose fermentation	*Geotrichum fermentans*: p. 576	→ 572
572	L-Sorbose growth	*Dipodascus macrosporus*: p. 189	*Dipodascus armillariae*: p. 184
573	Ethanol growth	→ 574	→ 577
574	Ribitol growth	→ 575	→ 576
575	Sucrose growth	*Aciculoconidium aculeatum*: p. 439	*Saccharomycopsis malanga*: p. 379
576	Soluble starch growth	*Pichia amylophila*: p. 285	*Pichia mississippiensis*: p. 325
577	L-Arabinose growth	*Protomyces inouyei*: p. 354	*Protomyces pachydermus*: p. 356
578	D-Xylose growth	*Dipodascus spicifer*: p. 191	→ 579
579	Galactose growth	*Geotrichum clavatum*: p. 575	*Eremothecium gossypii*: p. 204
580	Galactose growth	→ 581	→ 596

#	Test	Positive	Negative
581	D-Xylose growth	→ 582	→ 590
582	Maltose growth	→ 583	→ 584
583	Trehalose growth	*Candida albicans*: p. 476	*Dipodascus geniculatus*: p. 187
584	Vitamin-free growth	→ 585	→ 586
585	L-Arabinose growth	*Cephaloascus fragrans*: p. 144	*Galactomyces geotrichum*: p. 210 *Geotrichum klebahnii*: p. 578
586	D-Mannitol growth	→ 587	→ 589
587	Citrate growth	*Dipodascus aggregatus*: p. 182 *Galactomyces citri-aurantii*: p. 209	→ 588
588	Growth at 37°C	*Dipodascus australiensis*: p. 185	*Dipodascus albidus*: p. 183
589	Ribitol growth	*Saccharomycopsis selenospora*: p. 381	*Galactomyces reessii*: p. 212
590	Sucrose growth	→ 591	→ 592
591	D-Mannitol growth	*Dipodascus magnusii*: p. 189	*Eremothecium cymbalariae*: p. 204
592	D-Mannitol growth	→ 593	→ 594
593	Glucose fermentation	*Geotrichum fragrans*: p. 577	*Dipodascus ambrosiae*: p. 184
594	Growth at 37°C	→ 595	*Dipodascus ovetensis*: p. 190 *Geotrichum fragrans*: p. 577
595	Glucose fermentation	*Dipodascus tetrasperma*: p. 192 *Geotrichum fragrans*: p. 577	*Dipodascus capitatus*: p. 186
596	Growth at 37°C	→ 597	→ 603
597	Raffinose growth	→ 598	*Eremothecium sinecaudum*: p. 205
598	Trehalose growth	→ 599	*Schizosaccharomyces japonicus*: p. 391
599	Ethanol growth	→ 600	→ 602
600	Glucose fermentation	*Eremothecium coryli*: p. 202	→ 601
601	α-Methyl-D-glucoside growth	*Eremothecium cymbalariae*: p. 204	*Eremothecium gossypii*: p. 204
602	α-Methyl-D-glucoside growth	*Eremothecium cymbalariae*: p. 204	*Eremothecium ashbyi*: p. 201 *Eremothecium gossypii*: p. 204
603	Ethanol growth	→ 604	→ 608
604	DL-Lactate growth	*Saccharomycopsis crataegensis*: p. 376	→ 605
605	Glucose fermentation	→ 606	→ 607
606	2-Keto-D-gluconate growth	*Saccharomycopsis vini*: p. 383	*Saccharomycopsis fermentans*: p. 377
607	*N*-Acetyl-D-glucosamine growth	*Saccharomycopsis javanensis*: p. 379	*Saccharomycopsis schoenii*: p. 380
608	D-Mannitol growth	*Protomyces pachydermus*: p. 356	*Candida amapae*: p. 479
609	Starch formation	→ 610	→ 623
610	Sucrose growth	→ 611	→ 617
611	Melibiose growth	→ 354	→ 612
612	D-Mannitol growth	→ 613	→ 615
613	Maltose growth	→ 614	*Lipomyces japonicus*: p. 249
614	Ethanol growth	*Myxozyma mucilagina*: p. 595	→ 577
615	Ribitol growth	*Myxozyma vanderwaltii*: p. 596	→ 616
616	Ethanol growth	*Zygozyma smithiae*: p. 434	*Schizosaccharomyces pombe*: p. 393
617	Ethanol growth	→ 618	→ 621
618	Ribitol growth	→ 619	→ 620
619	D-Mannitol growth	*Zygozyma suomiensis*: p. 435	*Myxozyma lipomycoides*: p. 594
620	Galactitol growth	*Zygozyma oligophaga*: p. 434	*Dipodascus ingens*: p. 188
621	Trehalose growth	*Zygozyma arxii*: p. 433	→ 622
622	Glucose fermentation	*Schizosaccharomyces octosporus*: p. 392	*Babjevia anomala*: p. 141
623	Sucrose growth	→ 624	→ 826
624	Glucose fermentation	→ 625	→ 800

#	Test	Positive	Negative
625	D-Mannitol growth	→ 626	→ 775
626	Ethanol growth	→ 627	→ 770
627	Maltose growth	→ 628	→ 753
628	Trehalose growth	→ 629	→ 746
629	Galactose growth	→ 630	→ 721
630	Cellobiose growth	→ 631	→ 696
631	2-Keto-D-gluconate growth	→ 632	→ 689
632	Melibiose growth	→ 633	→ 638
633	10% NaCl/5% glucose growth	→ 634	*Debaryomyces occidentalis* var. *occidentalis*: p. 165
634	Ribitol growth	→ 635	*Zygosaccharomyces fermentati*: p. 427
635	L-Arabinose growth	→ 636	→ 637
636	Growth at 37°C	*Debaryomyces hansenii* var. *fabryi*: p. 162 *Pichia guilliermondii*: p. 308	*Debaryomyces castellii*: p. 159 *Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162
637	DL-Lactate growth	*Candida salmanticensis*: p. 548	*Candida melibiosica*: p. 524
638	Pellicle formation	→ 639	→ 647
639	Raffinose growth	→ 640	→ 645
640	D-Xylose growth	→ 641	*Pichia ohmeri*: p. 329
641	L-Arabinitol growth	→ 642	→ 643
642	Vitamin-free growth	*Debaryomyces vanrijiae* var. *yarrowii*: p. 170	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162
643	Galactose fermentation	*Candida intermedia*: p. 516 *Debaryomyces hansenii* var. *hansenii*: p. 161	→ 644
644	Vitamin-free growth	*Debaryomyces vanrijiae* var. *yarrowii*: p. 170	*Debaryomyces hansenii* var. *hansenii*: p. 161
645	Galactose fermentation	→ 646	*Candida tanzawaensis*: p. 561
646	Melezitose growth	*Candida oleophila*: p. 533	*Pichia segobiensis*: p. 340
647	Vitamin-free growth	→ 648	→ 649
648	L-Arabinose growth	*Candida kruisii*: p. 518	*Candida sake*: p. 547
649	Galactitol growth	→ 650	→ 653
650	D-Gluconate growth	→ 651	*Candida shehatae* var. *lignosa*: p. 552
651	Raffinose growth	→ 652	*Debaryomyces yamadae*: p. 171
652	L-Arabinose growth	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	*Candida pseudointermedia*: p. 541
653	Soluble starch growth	→ 654	→ 666
654	Inulin growth	→ 655	→ 656
655	10% NaCl/5% glucose growth	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	*Debaryomyces occidentalis* var. *occidentalis*: p. 165
656	Growth at 37°C	→ 657	→ 659
657	Propane 1,2 diol growth	*Candida fluviatilis*: p. 505	→ 658
658	Raffinose growth	*Debaryomyces hansenii* var. *fabryi*: p. 162	*Candida viswanathii*: p. 570
659	DL-Lactate growth	→ 660	→ 662
660	D-Gluconate growth	→ 661	*Candida shehatae* var. *lignosa*: p. 552
661	10% NaCl/5% glucose growth	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	*Candida tenuis*: p. 561
662	Gelatin liquefaction	→ 663	→ 665
663	L-Rhamnose growth	→ 661	→ 664
664	L-Arabinose growth	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	*Candida shehatae* var. *insectosa*: p. 552
665	L-Rhamnose growth	→ 661	*Candida shehatae* var. *shehatae*: p. 552 *Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162

#	Test	Positive	Negative
666	*N*-Acetyl-D-glucosamine growth	→ 667	→ 687
667	Growth at 37°C	→ 668	→ 675
668	α-Methyl-D-glucoside growth	→ 669	→ 673
669	L-Arabinose growth	→ 670	→ 672
670	Raffinose growth	*Debaryomyces hansenii* var. *fabryi*: p. 162	→ 671
671	D-Glucosamine growth	*Debaryomyces etchellsii*: p. 160	*Clavispora lusitaniae*: p. 148
672	0.1% Cycloheximide growth	*Candida maltosa*: p. 522	*Clavispora lusitaniae*: p. 148 *Metschnikowia pulcherrima*: p. 264
673	DL-Lactate growth	→ 674	*Metschnikowia agaves*: p. 257
674	50% Glucose growth	*Clavispora lusitaniae*: p. 148	*Clavispora opuntiae*: p. 150
675	10% NaCl/5% glucose growth	→ 676	→ 684
676	Hexadecane growth	→ 677	→ 680
677	L-Sorbose growth	→ 678	→ 679
678	Raffinose growth	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	*Candida sake*: p. 547 *Metschnikowia pulcherrima*: p. 264 *Metschnikowia reukaufii*: p. 265
679	Raffinose growth	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	*Metschnikowia lunata*: p. 263 *Metschnikowia reukaufii*: p. 265
680	α-Methyl-D-glucoside growth	→ 681	→ 683
681	Raffinose growth	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	→ 682
682	Galactose fermentation	*Candida sake*: p. 547	*Metschnikowia bicuspidata* var. *californica*: p. 259
683	Galactose fermentation	*Candida sake*: p. 547	*Metschnikowia bicuspidata* var. *bicuspidata*: p. 259
684	Melezitose growth	→ 685	*Pichia segobiensis*: p. 340
685	Lactose growth	*Candida tenuis*: p. 561	→ 686
686	0.01% Cycloheximide growth	*Candida natalensis*: p. 530	*Candida sake*: p. 547
687	Raffinose growth	→ 688	*Candida sake*: p. 547
688	Ribitol growth	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162	*Zygosaccharomyces fermentati*: p. 427
689	D-Glucosamine growth	→ 690	→ 691
690	Ribitol growth	*Metschnikowia zobellii*: p. 266	*Candida buinensis*: p. 491
691	L-Rhamnose growth	*Pichia strasburgensis*: p. 343	→ 692
692	Lactose growth	→ 693	→ 694
693	Melezitose growth	*Kluyveromyces lactis* var. *lactis*: p. 233	*Kluyveromyces aestuarii*: p. 229
694	Galactose fermentation	*Kluyveromyces dobzhanskii*: p. 233 *Kluyveromyces lactis* var. *drosophilarum*: p. 234	→ 695
695	α-Methyl-D-glucoside growth	*Metschnikowia bicuspidata* var. *chathamia*: p. 259	*Metschnikowia agaves*: p. 257
696	L-Rhamnose growth	→ 697	→ 698
697	L-Sorbose growth	*Debaryomyces vanrijiae* var. *yarrowii*: p. 170	*Candida haemulonii*: p. 511
698	L-Arabinose growth	→ 699	→ 702
699	Hexadecane growth	→ 700	→ 701
700	Galactitol growth	*Debaryomyces vanrijiae* var. *yarrowii*: p. 170	*Candida parapsilosis*: p. 536
701	Galactose fermentation	*Kluyveromyces lactis* var. *drosophilarum*: p. 234	*Candida mogii*: p. 527
702	Raffinose growth	→ 703	→ 714
703	0.01% Cycloheximide growth	→ 704	→ 708
704	10% NaCl/5% glucose growth	→ 705	→ 707
705	Melezitose growth	→ 706	*Zygosaccharomyces cidri*: p. 426
706	Ribitol growth	*Debaryomyces vanrijiae* var. *yarrowii*: p. 170	*Zygosaccharomyces fermentati*: p. 427
707	Melibiose growth	*Zygosaccharomyces florentinus*: p. 428	*Kluyveromyces lactis* var. *drosophilarum*: p. 234
708	Vitamin-free growth	→ 709	→ 711

#	Test	Positive	Negative
709	Galactitol growth	*Debaryomyces vanrijiae* var. *yarrowii*: p. 170	→ 710
710	Inulin growth	*Torulaspora delbrueckii*: p. 404 *Torulaspora pretoriensis*: p. 406	*Saccharomyces bayanus*: p. 360 *Torulaspora delbrueckii*: p. 404
711	DL-Lactate growth	→ 712	→ 713
712	Melibiose growth	*Saccharomyces kluyveri*: p. 365	*Saccharomyces paradoxus*: p. 366 *Torulaspora delbrueckii*: p. 404
713	10% NaCl/5% glucose growth	*Torulaspora delbrueckii*: p. 404	*Kluyveromyces thermotolerans*: p. 240
714	Succinate growth	→ 715	→ 720
715	Growth at 37°C	→ 716	→ 718
716	*N*-Acetyl-D-glucosamine growth	*Lodderomyces elongisporus*: p. 254	→ 717
717	2-Keto-D-gluconate growth	*Torulaspora delbrueckii*: p. 404	*Kluyveromyces lactis* var. *drosophilarum*: p. 234
718	D-Glucosamine growth	*Candida quercitrusa*: p. 543 *Candida sake*: p. 547	→ 719
719	Cadaverine growth	*Candida sake*: p. 547	*Torulaspora delbrueckii*: p. 404
720	2-Keto-D-gluconate growth	*Torulaspora delbrueckii*: p. 404	*Zygosaccharomyces rouxii*: p. 431
721	Citrate growth	→ 722	→ 741
722	D-Xylose growth	→ 723	→ 739
723	DL-Lactate growth	→ 724	→ 736
724	*N*-Acetyl-D-glucosamine growth	*Pichia toletana*: p. 346 *Pichia xylosa*: p. 350	→ 725
725	Growth at 37°C	→ 726	→ 733
726	Raffinose growth	→ 727	→ 729
727	Ribitol growth	→ 728	*Pichia onychis*: p. 330
728	10% NaCl/5% glucose growth	*Pichia rabaulensis*: p. 337	*Candida odintsovae*: p. 532
729	Melezitose growth	→ 730	*Pichia meyerae*: p. 323
730	2-Keto-D-gluconate growth	*Pichia wickerhamii*: p. 349	→ 731
731	L-Arabinose growth	*Pichia bovis*: p. 292	→ 732
732	Gelatin liquefaction	*Candida freyschussii*: p. 506 *Pichia japonica*: p. 315	*Candida freyschussii*: p. 506 *Pichia rhodanensis*: p. 338
733	Ribitol growth	*Pichia hampshirensis*: p. 309	→ 734
734	L-Rhamnose growth	→ 735	*Candida quercuum*: p. 543
735	Pellicle formation	*Pichia veronae*: p. 348	*Candida maritima*: p. 523
736	L-Sorbose growth	→ 737	→ 738
737	Melezitose growth	*Candida musae*: p. 528	*Candida suecica*: p. 560
738	L-Arabinose growth	*Pichia angophorae*: p. 286	*Candida oregonensis*: p. 534
739	Galactose fermentation	*Debaryomyces occidentalis* var. *persoonii*: p. 165	→ 740
740	Cellobiose growth	*Pichia spartinae*: p. 341	*Candida tsuchiyae*: p. 564
741	Cellobiose growth	→ 742	→ 745
742	Ribitol growth	*Metschnikowia reukaufii*: p. 265	→ 743
743	DL-Lactate growth	→ 744	*Williopsis mucosa*: p. 415
744	L-Rhamnose growth	*Pichia xylosa*: p. 350	*Candida solani*: p. 555
745	Raffinose growth	*Saccharomyces bayanus*: p. 360 *Torulaspora delbrueckii*: p. 404	→ 720
746	Lactose growth	*Kluyveromyces aestuarii*: p. 229	→ 747
747	Galactose fermentation	→ 748	→ 749
748	0.01% Cycloheximide growth	*Kluyveromyces lactis* var. *drosophilarum*: p. 234	*Nadsonia fulvescens* var. *fulvescens*: p. 269
749	Ribitol growth	→ 750	→ 751
750	Galactitol growth	*Debaryomyces vanrijiae* var. *yarrowii*: p. 170	*Pichia wickerhamii*: p. 349
751	Cellobiose growth	→ 752	*Candida floricola*: p. 505

#	Test	Positive	Negative
752	L-Sorbose growth	*Metschnikowia gruessii*: p. 260	*Pichia xylosa*: p. 350
753	Galactitol growth	*Candida spandovensis*: p. 558	→ 754
754	Melibiose growth	→ 755	→ 756
755	Trehalose growth	*Zygosaccharomyces microellipsoides*: p. 430	*Zygosaccharomyces mrakii*: p. 430
756	*N*-Acetyl-D-glucosamine growth	→ 757	→ 758
757	Galactose fermentation	*Wickerhamia fluorescens*: p. 409	*Candida apicola*: p. 481
758	Hexadecane growth	*Candida gropengiesseri*: p. 510	→ 759
759	Succinate growth	→ 760	→ 765
760	10% NaCl/5% glucose growth	→ 761	→ 763
761	Cellobiose growth	*Kluyveromyces aestuarii*: p. 229	→ 762
762	Trehalose growth	*Torulaspora delbrueckii*: p. 404	*Candida bombicola*: p. 490
763	Melezitose growth	→ 764	*Kluyveromyces lactis* var. *drosophilarum*: p. 234 *Kluyveromyces marxianus*: p. 236
764	Lactose growth	*Kluyveromyces lactis* var. *lactis*: p. 233	*Kluyveromyces lactis* var. *drosophilarum*: p. 234
765	D-Xylose growth	→ 766	→ 767
766	Trehalose growth	*Torulaspora delbrueckii*: p. 404	*Kluyveromyces waltii*: p. 241
767	Raffinose growth	→ 768	→ 769
768	0.01% Cycloheximide growth	*Torulaspora globosa*: p. 406	*Torulaspora delbrueckii*: p. 404
769	Maltose fermentation	→ 720	*Torulaspora delbrueckii*: p. 404 *Zygosaccharomyces bailii*: p. 424
770	Trehalose growth	→ 771	→ 772
771	L-Sorbose growth	*Candida bombi*: p. 489	*Zygosaccharomyces rouxii*: p. 431
772	Raffinose growth	→ 773	*Candida geochares*: p. 508
773	Galactose fermentation	*Zygosaccharomyces mrakii*: p. 430	→ 774
774	Galactose growth	*Candida gropengiesseri*: p. 510	*Candida apicola*: p. 481
775	Ribitol growth	→ 776	→ 779
776	Ethanol growth	→ 777	*Candida multigemmis*: p. 528
777	Melibiose growth	*Candida xestobii*: p. 571	→ 778
778	Galactose fermentation	*Kluyveromyces lactis* var. *drosophilarum*: p. 234 *Kluyveromyces marxianus*: p. 236	*Pichia hampshirensis*: p. 309
779	D-Xylose growth	→ 780	→ 782
780	Lactose growth	*Kluyveromyces wickerhamii*: p. 242	→ 781
781	Galactose fermentation	*Kluyveromyces lactis* var. *drosophilarum*: p. 234	*Pichia meyerae*: p. 323
782	D-Glucosamine growth	*Dekkera anomala*: p. 174 *Dekkera bruxellensis*: p. 175	→ 783
783	Raffinose fermentation	→ 784	→ 795
784	Vitamin-free growth	*Saccharomyces bayanus*: p. 360	→ 785
785	Maltose growth	→ 786	→ 788
786	Ethanol growth	→ 787	*Schizosaccharomyces pombe*: p. 393
787	Cadaverine growth	*Kluyveromyces lactis* var. *drosophilarum*: p. 234	*Saccharomyces cerevisiae*: p. 361 *Saccharomyces pastorianus*: p. 367
788	Galactose fermentation	→ 789	→ 793
789	D-Gluconate growth	→ 790	→ 791
790	Citrate growth	*Kluyveromyces polysporus*: p. 239	*Saccharomyces barnettii*: p. 359
791	Cadaverine growth	→ 792	*Candida milleri*: p. 526 *Saccharomyces exiguus*: p. 364
792	Growth at 37°C	*Kluyveromyces lactis* var. *drosophilarum*: p. 234	*Kluyveromyces lodderae*: p. 235
793	Ethanol growth	*Saccharomycodes ludwigii*: p. 372	→ 794
794	Trehalose growth	*Cyniclomyces guttulatus*: p. 154	*Candida stellata*: p. 559

#	Test	Positive	Negative
795	Succinate growth	→ 796	→ 797
796	Ethanol growth	*Kluyveromyces lactis* var. *drosophilarum*: p. 234	*Saccharomyces spencerorum*: p. 368
797	D-Gluconate growth	*Kluyveromyces bacillisporus*: p. 230	→ 798
798	Cadaverine growth	→ 799	*Dekkera bruxellensis*: p. 175
799	Maltose growth	*Hanseniaspora osmophila*: p. 216 *Hanseniaspora vineae*: p. 219	*Hanseniaspora occidentalis*: p. 215
800	Galactose growth	→ 801	→ 819
801	Ribitol growth	→ 802	→ 813
802	Pellicle formation	→ 803	→ 805
803	D-Gluconate growth	→ 804	*Debaryomyces carsonii*: p. 158
804	Growth at 40°C	*Candida palmioleophila*: p. 535	→ 642
805	Cellobiose growth	→ 806	→ 812
806	10% NaCl/5% glucose growth	→ 807	→ 810
807	Propane 1,2 diol growth	→ 808	→ 809
808	DL-Lactate growth	*Candida saitoana*: p. 546	*Lipomyces starkeyi*: p. 252
809	Vitamin-free growth	*Lipomyces starkeyi*: p. 252	*Debaryomyces hansenii* var. *hansenii*: p. 161 *Debaryomyces hansenii* var. *fabryi*: p. 162
810	Melibiose growth	→ 811	*Candida tenuis*: p. 561
811	DL-Lactate growth	*Candida glaebosa*: p. 509	*Lipomyces starkeyi*: p. 252
812	Melibiose growth	*Lipomyces starkeyi*: p. 252	*Candida azyma*: p. 485
813	DL-Lactate growth	→ 814	→ 816
814	D-Mannitol growth	→ 815	*Candida caseinolytica*: p. 492
815	Trehalose growth	*Candida tepae*: p. 562	*Candida antillancae*: p. 481
816	Melibiose growth	→ 817	→ 818
817	Growth at 40°C	*Lipomyces kononenkoae* ssp. *spencer-martinsiae*: p. 250	*Lipomyces kononenkoae* ssp. *kononenkoae*: p. 250 *Lipomyces starkeyi*: p. 252
818	Maltose growth	*Metschnikowia australis*: p. 258	*Candida apis*: p. 482
819	DL-Lactate growth	→ 820	→ 823
820	L-Sorbose growth	→ 821	→ 822
821	Ribitol growth	*Candida petrohuensis*: p. 539	*Candida ancudensis*: p. 480
822	*N*-Acetyl-D-glucosamine growth	*Pichia xylosa*: p. 350	*Pichia canadensis*: p. 295
823	0.01% Cycloheximide growth	→ 824	→ 825
824	Melibiose growth	*Lipomyces starkeyi*: p. 252	*Candida drimydis*: p. 500
825	Ribitol growth	*Candida suecica*: p. 560	*Metschnikowia krissii*: p. 262
826	L-Rhamnose growth	→ 827	→ 839
827	Glucose fermentation	→ 828	→ 835
828	Galactitol growth	*Pichia tannicola*: p. 345	→ 829
829	Cellobiose growth	→ 830	*Pichia pastoris*: p. 331
830	Pellicle formation	*Williopsis saturnus* var. *sargentensis*: p. 417	→ 831
831	Succinate growth	→ 832	→ 834
832	D-Xylose growth	→ 833	*Candida anatomiae*: p. 479
833	Citrate growth	*Pichia minuta* var. *minuta*: p. 324	*Brettanomyces naardenensis*: p. 451
834	Galactose fermentation	*Brettanomyces nanus*: p. 452	*Pichia minuta* var. *minuta*: p. 324
835	Ribitol growth	*Candida maris*: p. 523	→ 836
836	D-Xylose growth	*Pichia salicaria*: p. 338	→ 837
837	Citrate growth	→ 838	*Pichia thermotolerans*: p. 346
838	Cellobiose growth	*Candida montana*: p. 527 *Pichia opuntiae*: p. 330	*Pichia antillensis*: p. 288 *Pichia opuntiae*: p. 330
839	Galactose fermentation	→ 840	→ 853

#	Test	Positive	Negative
840	Citrate growth	→ 841	→ 842
841	Cellobiose growth	*Candida savonica*: p. 550	*Candida catenulata*: p. 494
842	0.01% Cycloheximide growth	→ 843	→ 846
843	Succinate growth	→ 844	→ 845
844	Cellobiose growth	*Brettanomyces naardenensis*: p. 451	*Saccharomyces unisporus*: p. 370
845	Growth at 37°C	*Dekkera bruxellensis*: p. 175	*Saccharomyces servazzii*: p. 368
846	Trehalose growth	→ 847	→ 850
847	D-Mannitol growth	*Torulaspora delbrueckii*: p. 404	→ 848
848	DL-Lactate growth	*Candida humilis*: p. 513	→ 849
849	Succinate growth	*Saccharomyces castellii*: p. 360 *Saccharomyces dairenensis*: p. 363	*Saccharomyces castellii*: p. 360 *Saccharomyces transvaalensis*: p. 369
850	D-Gluconate growth	*Kluyveromyces phaffii*: p. 239	→ 851
851	Ethanol growth	*Kluyveromyces yarrowii*: p. 243	→ 852
852	2-Keto-D-gluconate growth	*Kluyveromyces blattae*: p. 231 *Saccharomyces rosinii*: p. 367	*Kluyveromyces africanus*: p. 230 *Saccharomyces rosinii*: p. 367
853	D-Mannitol growth	→ 854	→ 899
854	Maltose fermentation	→ 855	→ 857
855	Citrate growth	*Candida catenulata*: p. 494	→ 856
856	2-Keto-D-gluconate growth	*Torulaspora delbrueckii*: p. 404	*Zygosaccharomyces mellis*: p. 429 *Zygosaccharomyces rouxii*: p. 431
857	Trehalose growth	→ 858	→ 878
858	Melezitose growth	→ 859	→ 860
859	Cellobiose growth	*Candida insectamans*: p. 515	*Torulaspora delbrueckii*: p. 404
860	Succinate growth	→ 861	→ 877
861	L-Sorbose growth	→ 862	→ 870
862	0.01% Cycloheximide growth	→ 863	→ 867
863	Citrate growth	→ 864	*Botryozyma nematodophila*: p. 449
864	D-Gluconate growth	→ 865	*Trigonopsis variabilis*: p. 605
865	D-Xylose growth	*Candida beechii*: p. 485	→ 866
866	Xylitol growth	*Candida santamariae*: p. 549	*Candida zeylanoides*: p. 571
867	Ethanol growth	→ 868	*Schizoblastosporion starkeyi-henricii*: p. 602
868	Cellobiose growth	*Candida torresii*: p. 562	→ 869
869	D-Glucosamine growth	*Candida fructus*: p. 507	*Torulaspora delbrueckii*: p. 404
870	Cellobiose growth	→ 871	→ 872
871	Glucose fermentation	→ 833	*Candida insectalens*: p. 514
872	Ethanol growth	→ 873	*Schizoblastosporion starkeyi-henricii*: p. 602
873	Citrate growth	*Candida catenulata*: p. 494	→ 874
874	Glucose fermentation	→ 875	*Candida silvatica*: p. 554
875	Gelatin liquefaction	*Saturnispora saitoi*: p. 389	→ 876
876	2-Keto-D-gluconate growth	*Torulaspora delbrueckii*: p. 404	*Saturnispora dispora*: p. 388 *Saturnispora zaruensis*: p. 390
877	Cellobiose growth	*Pichia minuta* var. *minuta*: p. 324	*Torulaspora delbrueckii*: p. 404 *Zygosaccharomyces bailii*: p. 424
878	Ethanol growth	→ 879	→ 897
879	D-Glucosamine growth	→ 880	→ 881
880	Xylitol growth	*Candida vinaria*: p. 568	*Candida krissii*: p. 517
881	Cellobiose growth	→ 882	→ 885
882	Ribitol growth	*Candida sonorensis*: p. 556	→ 883
883	L-Sorbose growth	*Pichia pijperi*: p. 334	→ 884
884	Glucose fermentation	*Pichia quercuum*: p. 337	*Pichia thermotolerans*: p. 346

#	Test	Positive	Negative
885	*N*-Acetyl-D-glucosamine growth	→ 886	→ 887
886	Galactose growth	*Candida rugosa*: p. 546	*Pichia delftensis*: p. 299
887	Glucose fermentation	→ 888	→ 894
888	L-Sorbose growth	→ 889	→ 890
889	DL-Lactate growth	*Candida cylindracea*: p. 497	*Zygosaccharomyces bisporus*: p. 426
890	Citrate growth	*Candida diversa*: p. 499 *Candida silvae*: p. 553	→ 891
891	D-Gluconate growth	→ 892	→ 893
892	Ribitol growth	*Candida silvae*: p. 553	*Pichia caribaea*: p. 297
893	Ribitol growth	*Candida silvae*: p. 553 *Candida vini*: p. 569	*Candida vini*: p. 569 *Pichia besseyi*: p. 290 *Saturnispora ahearnii*: p. 387
894	Galactose growth	→ 895	→ 896
895	DL-Lactate growth	*Candida pararugosa*: p. 538	*Candida sorbophila*: p. 557
896	D-Gluconate growth	*Pichia amethionina* var. *pachycereana*: p. 284	*Pichia fluxuum*: p. 306
897	Glucose fermentation	*Candida karawaiewii*: p. 517	→ 898
898	Citrate growth	*Candida galacta*: p. 507	*Schizoblastosporion starkeyi-henricii*: p. 602
899	Cellobiose growth	→ 900	→ 911
900	Glucose fermentation	→ 901	→ 908
901	Succinate growth	→ 902	→ 904
902	Trehalose growth	*Brettanomyces naardenensis*: p. 451	→ 903
903	D-Glucosamine growth	*Pichia norvegensis*: p. 328	*Candida dendrica*: p. 497
904	Growth at 37°C	→ 905	→ 906
905	Cadaverine growth	*Hanseniaspora guilliermondii*: p. 215	*Dekkera bruxellensis*: p. 175
906	Maltose growth	*Hanseniaspora osmophila*: p. 216 *Hanseniaspora vineae*: p. 219	→ 907
907	2-Keto-D-gluconate growth	*Hanseniaspora uvarum*: p. 217	*Hanseniaspora valbyensis*: p. 218 *Kloeckera lindneri*: p. 581
908	D-Xylose growth	*Babjevia anomala*: p. 141	→ 909
909	Galactose growth	→ 910	→ 269
910	Maltose growth	*Prototheca stagnora*: p. 885	*Pichia chambardii*: p. 298
911	Maltose growth	→ 912	→ 918
912	Glucose fermentation	→ 913	→ 914
913	0.01% Cycloheximide growth	*Dekkera bruxellensis*: p. 175	*Schizosaccharomyces octosporus*: p. 392
914	Hexadecane growth	*Prototheca zopfii* var. *hydrocarbonea*: p. 886	→ 915
915	Succinate growth	→ 916	*Prototheca moriformis*: p. 884 *Prototheca zopfii* var. *zopfii*: p. 886 *Prototheca zopfii* var. *portoricensis*: p. 886
916	Galactose growth	*Prototheca stagnora*: p. 885	→ 917
917	Growth at 25°C	*Prototheca moriformis*: p. 884	*Nadsonia commutata*: p. 268
918	Galactose growth	→ 919	→ 926
919	Growth at 25°C	→ 920	*Candida austromarina*: p. 484
920	Vitamin-free growth	*Dipodascus ingens*: p. 188	→ 921
921	Ethanol growth	→ 922	→ 925
922	Trehalose growth	→ 923	→ 924
923	Glucose fermentation	*Dekkera bruxellensis*: p. 175	*Candida caseinolytica*: p. 492 *Prototheca wickerhamii*: p. 886
924	Xylitol growth	*Candida vinaria*: p. 568	*Dekkera bruxellensis*: p. 175
925	Glucose fermentation	*Dekkera bruxellensis*: p. 175	*Schizoblastosporion starkeyi-henricii*: p. 602
926	D-Glucosamine growth	→ 927	→ 941

#	Test	Positive	Negative
927	Pellicle formation	→ 928	→ 939
928	Vitamin-free growth	→ 929	→ 931
929	Citrate growth	*Issatchenkia orientalis*: p. 222	→ 930
		Pichia membranifaciens: p. 319	
930	Glycerol growth	*Issatchenkia occidentalis*: p. 221	*Candida rugopelliculosa*: p. 545
		Pichia membranifaciens: p. 319	*Pichia membranifaciens*: p. 319
931	*N*-Acetyl-D-glucosamine growth	→ 932	→ 937
932	Succinate growth	→ 933	→ 936
933	Glucose fermentation	→ 934	*Pichia kluyveri* var. *eremophila*: p. 316
			Pichia membranifaciens: p. 319
			Prototheca ulmea: p. 885
934	Citrate growth	→ 935	*Pichia barkeri*: p. 289
			Pichia kluyveri var. *kluyveri*: p. 315
			Pichia membranifaciens: p. 319
935	D-Xylose growth	*Pichia fermentans*: p. 305	*Pichia kluyveri* var. *kluyveri*: p. 315
		Pichia kluyveri var. *kluyveri*: p. 315	*Pichia kluyveri* var. *cephalocereana*: p. 316
		Pichia membranifaciens: p. 319	*Pichia kluyveri* var. *eremophila*: p. 316
			Pichia membranifaciens: p. 319
936	Glucose fermentation	*Dekkera bruxellensis*: p. 175	*Pichia membranifaciens*: p. 319
		Pichia membranifaciens: p. 319	*Prototheca ulmea*: p. 885
937	D-Xylose growth	*Pichia cactophila*: p. 294	→ 938
938	Glucose fermentation	*Dekkera bruxellensis*: p. 175	*Pichia pseudocactophila*: p. 336
			Prototheca ulmea: p. 885
939	Glucose fermentation	→ 940	*Candida inconspicua*: p. 514
			Prototheca ulmea: p. 885
940	D-Xylose growth	*Candida pseudolambica*: p. 542	*Dekkera bruxellensis*: p. 175
941	L-Sorbose growth	→ 942	→ 946
942	Ethanol growth	→ 943	*Prototheca ulmea*: p. 885
			Schizoblastosporion starkeyi-henricii: p. 602
943	*N*-Acetyl-D-glucosamine growth	*Pichia membranifaciens*: p. 319	→ 944
		Prototheca ulmea: p. 885	
944	D-Xylose growth	*Candida sorboxylosa*: p. 558	→ 945
945	Glucose fermentation	*Nadsonia fulvescens* var. *elongata*: p. 269	*Prototheca ulmea*: p. 885
946	Vitamin-free growth	→ 947	→ 950
947	Growth at 37°C	*Candida ethanolica*: p. 503	→ 948
		Pichia galeiformis: p. 307	
		Pichia membranifaciens: p. 319	
948	*N*-Acetyl-D-glucosamine growth	→ 949	*Issatchenkia scutulata* var. *scutulata*: p. 224
949	10% NaCl/5% glucose growth	*Pichia membranifaciens*: p. 319	*Issatchenkia scutulata* var. *exigua*: p. 224
950	Glucose fermentation	→ 951	→ 961
951	Glycerol growth	→ 952	→ 958
952	Succinate growth	→ 953	→ 955
953	Trehalose growth	*Brettanomyces custersianus*: p. 451	→ 954
954	*N*-Acetyl-D-glucosamine growth	*Pichia membranifaciens*: p. 319	*Issatchenkia terricola*: p. 225
			Pichia nakasei: p. 326
955	D-Gluconate growth	→ 956	→ 957
956	Cadaverine growth	*Candida glabrata*: p. 508	*Candida castellii*: p. 493
957	Ethanol growth	*Dekkera bruxellensis*: p. 175	→ 913
		Pichia membranifaciens: p. 319	
958	D-Gluconate growth	*Kluyveromyces delphensis*: p. 232	→ 959
959	*N*-Acetyl-D-glucosamine growth	*Dekkera bruxellensis*: p. 175	→ 960
		Pichia membranifaciens: p. 319	

#	Test	Positive	Negative
960	Growth at 37°C	*Arxiozyma telluris*: p. 134 *Dekkera bruxellensis*: p. 175	*Schizosaccharomyces octosporus*: p. 392
961	Growth at 25°C	→ 962	*Nadsonia commutata*: p. 268
962	Ethanol growth	→ 963	*Prototheca ulmea*: p. 885 *Schizoblastosporion starkeyi-henricii*: p. 602
963	D-Gluconate growth	*Pichia amethionina* var. *amethionina*: p. 284 *Prototheca ulmea*: p. 885	→ 964
964	D-Xylose growth	→ 965	→ 966
965	*N*-Acetyl-D-glucosamine growth	*Pichia membranifaciens*: p. 319	*Pichia heedii*: p. 310
966	*N*-Acetyl-D-glucosamine growth	*Pichia membranifaciens*: p. 319 *Prototheca ulmea*: p. 885	*Pichia deserticola*: p. 300 *Prototheca ulmea*: p. 885

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Summary of species characteristics

Contents

Introductory remarks

Taxa are given in alphabetical order regardless of their phylogenetic relationships. The species listed are those that readily grow in standard media used for fermentation and assimilation tests. Consequently, *Coccidiascus*, *Endomyces*, Endomycete-like species and *Malassezia* are excluded. Data for *Lalaria* species are limited and members of this genus are omitted from the summary. *Fellomyces horovitziae* is characterized in the treatment of the genus *Fellomyces*, but the data were received too late for inclusion here. Assimilation reactions for *Aureobasidium pullulans* are given here but not elsewhere in the book.

When sexual states are known for common anamorphic species, the taxon is listed in the summary under the teleomorphic name. For example, *Candida lipolytica* is given under *Yarrowia lipolytica* and *Phaffia rhodozyma* is given as *Xanthophyllomyces dendrorhous.* Known anamorph–teleomorph connections can be made by consulting the indexes.

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Aciculoconidium																																			
1. *A. aculeatum*	v	–	v	–	–	–	w	+	–	–	+	+	+	+	–	–	–	+	–	×	–	–	–	–	–	v	n	–	+	+	–	+	–	+	+
Agaricostilbum																																			
2. *A. hyphaenes*	–	–	–	–	–	–	–	+	–	+	+	–	+	+	+	–	+	+	–	–	+	+	+	+	–	–	n	–	+	+	+	+	–	+	+
Ambrosiozyma																																			
3. *A. ambrosiae*	v	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	v	+	–	–	v	–	–	n	–	v	+	+	+	–	+	+
4. *A. cicatricosa*	+	–	v	+	–	–	v	+	–	–	+	+	+	+	–	–	–	+	–	–	+	+	–	v	–	–	n	–	+	+	+	+	–	+	+
5. *A. monospora*	+	–	v	v	–	–	+	+	–	–	+	+	+	+	–	–	–	+	–	–	+	+	–	v	–	v	n	–	+	+	+	+	–	+	+
6. *A. philentoma*	+	–	v	–	–	–	+	+	–	–	+	+	+	+	–	–	–	+	–	–	+	–	–	+	+	+	n	–	+	+	+	+	–	+	+
7. *A. platypodis*	+	–	–	v	–	–	v	+	–	–	+	+	+	+	–	–	–	+	–	v	+	–	–	+	+	+	n	–	+	+	+	+	–	+	+
Arxiozyma																																			
8. *A. telluris*	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	–	–
Arxula																																			
9. *A. adeninivorans*	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	n	–	+	+	+	+	–	+	+
10. *A. terrestris*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	–	–	+	+	+	+	+	+	–	n	–	+	+	+	+	+	+	+
Ascoidea																																			
11. *A. africana*	–	–	–	–	–	–	–	+	+	+	–	–	w	+	–	+	+	w	–	+	+	+	–	–	–	w	n	w	+	+	–	+	n	+	+
12. *A. corymbosa*	–	–	–	–	–	–	–	+	–	w	+	+	+	w	–	v	–	+	–	+	+	v	–	–	+	w	n	w	+	+	–	–	n	–	+
13. *A. hylecoeti*	–	–	–	–	–	–	–	+	–	+	–	–	+	+	–	+	–	w	–	+	+	+	–	–	+	+	n	w	+	+	–	w	n	w	+
14. *A. rubescens*	–	–	–	–	–	–	–	+	w	–	+	+	+	+	+	w	+	w	w	+	+	–	+	+	+	–	n	–	+	w	+	+	n	+	w
Aureobasidium																																			
15. *A. pullulans*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	v	+	+	+	+	+	+	+	×	+	+	×	n	n	+	+	+	+	v	+	+
Babjevia																																			
16. *B. anomala*	–	–	–	–	–	–	–	+	v	v	–	v	+	–	+	–	–	–	–	–	+	–	–	–	–	–	n	–	–	v	–	v	–	–	–
Bensingtonia																																			
17. *B. ciliata*	–	–	–	–	–	–	–	+	–	w	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	n	–	+	+	–	+	–	+	+
18. *B. ingoldii*	–	–	–	–	–	–	–	+	–	–	+	–	+	+	+	+	+	+	–	+	+	+	–	+	–	–	n	–	+	+	+	+	–	+	+
19. *B. intermedia*	–	–	–	–	–	–	–	+	v	+	v	v	+	+	–	–	–	v	–	–	v	–	+	+	–	–	n	–	+	+	–	+	–	+	+
20. *B. miscanthi*	–	–	–	–	–	–	–	+	–	+	+	–	+	+	+	–	+	–	–	–	+	–	–	–	–	–	n	–	+	+	–	+	–	+	+
21. *B. naganoensis*	–	–	–	–	–	–	–	+	–	–	+	+	+	–	–	+	+	+	–	+	–	–	–	–	–	–	n	–	–	+	–	+	–	+	+
22. *B. phyllada*	–	–	–	–	–	–	–	+	–	+	+	+	+	+	w	–	–	+	–	+	–	w	–	–	w	–	n	–	+	+	–	+	–	+	+
23. *B. subrosea*	–	–	–	–	–	–	–	+	–	+	+	+	+	+	+	–	+	–	–	+	+	–	–	–	–	–	n	–	+	+	–	+	–	+	+
24. *B. yamatoana*	–	–	–	–	–	–	–	+	–	–	+	+	–	+	–	–	–	+	–	v	+	–	–	–	–	–	n	–	+	+	–	w	–	+	+
25. *B. yuccicola*	–	–	–	–	–	–	–	+	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	n	–	–	+	–	+	–	+	+
Blastobotrys																																			
26. *B. arbuscula*	+	+	–	+	–	–	+	+	+	+	–	+	+	+	–	–	–	–	v	+	+	+	–	+	–	+	n	n	–	+	+	+	+	+	+
27. *B. aristata*	+	v	–	+	–	–	+	+	+	+	+	+	+	+	+	v	v	–	v	+	+	+	+	+	v	+	n	n	–	+	+	+	+	+	+
28. *B. capitulata*	+	+	–	v	–	–	v	+	+	+	v	v	+	+	v	–	v	–	v	+	+	+	v	+	+	+	n	n	+	+	+	+	+	+	+
29. *B. elegans*	–	–	–	–	–	–	–	+	+	v	–	+	+	+	+	–	–	–	v	v	+	v	–	+	–	v	n	n	+	+	+	+	v	+	+
30. *B. nivea*	+	+	–	+	–	–	+	+	+	+	v	+	+	+	+	+	–	–	v	+	+	+	+	+	–	+	n	n	+	+	+	+	+	+	+
31. *B. proliferans*	+	+	+	+	–	+	v	+	+	+	+	+	+	+	+	+	+	v	v	v	+	+	+	v	v	+	n	n	+	+	+	+	+	+	+
Botryozyma																																			
32. *B. nematodophila*	–	–	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	n	n	+	+	–	–	–	+	+
Brettanomyces																																			
33. *B. custersianus*	+	–	–	–	–	–	v	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	n	–	+	+	–	–	–	–	–
34. *B. naardenensis*	+	v	–	–	–	–	v	+	v	–	–	v	+	+	–	–	–	–	–	v	+	–	v	–	v	v	n	–	+	–	–	v	–	v	+
35. *B. nanus*	+	+	–	–	–	–	–	+	+	–	–	–	+	v	v	–	–	–	–	–	v	–	–	–	+	–	n	–	+	–	–	+	–	+	+
Bullera																																			
36. *B. armeniaca*	–	–	–	–	–	–	–	+	+	–	+	+	+	+	–	–	+	+	–	–	+	+	+	+	+	+	n	–	–	+	–	+	+	+	+
37. *B. crocea*	–	–	–	–	–	–	–	+	+	–	+	+	+	+	–	+	+	+	–	w	+	+	+	+	+	–	n	–	–	v	–	+	+	+	+
38. *B. dendrophila*	–	–	–	–	–	–	–	+	+	–	+	+	+	+	+	–	–	+	–	+	+	+	+	+	+	–	n	–	+	+	–	+	+	+	+
39. *B. globispora*	–	–	–	–	–	–	–	+	+	–	+	+	+	+	–	–	+	–	–	v	+	+	+	+	+	–	n	–	–	+	–	+	+	+	+
40. *B. megalospora*	–	–	–	–	–	–	–	+	w	w	+	+	+	+	–	–	+	+	–	+	+	+	v	+	–	–	n	–	+	+	–	+	–	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

	Assimilation reactions and other characteristics																																							
	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Aciculoconidium																																								
1.	+	v	−	−	+	+	−	n	−	n	−	+	−	n	−	−	v	−	−	+	−	+	+	−	n	−	−	n	+	+	+	+	n	−	−	9	40.9	−	n	+
Agaricostilbum																																								
2.	+	+	+	−	+	+	−	n	−	−	−	+	n	n	n	n	n	n	n	n	n	n	n	+	n	−	+	n	n	n	+	+	−	−	−	n	n	+	−	v
Ambrosiozyma																																								
3.	+	+	−	v	+	+	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	+	+	+	+	v	−	−	7	34.7	−	−	+
4.	+	v	−	+	+	+	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	+	+	+	+	+	+	n	n	40.2	−	−	+
5.	+	+	−	+	+	v	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	+	+	+	+	+	+	n	7	38.9	−	−	+
6.	+	+	−	+	+	+	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	+	+	+	+	n	−	−	7	35.1	−	−	+
7.	+	+	−	v	+	+	−	n	+	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	+	+	+	+	n	−	−	7	37.8	−	−	+
Arxiozyma																																								
8.	−	−	−	v	v	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	v	n	n	n	v	+	+	n	6	33.2	−	−	−
Arxula																																								
9.	+	+	+	−	+	+	+	n	+	n	−	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	n	+	+	+	+	+	+	+	9	n	−	+	+
10.	+	+	+	+	+	+	+	n	+	n	−	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	n	+	+	+	+	+	+	−	9	n	−	−	+
Ascoidea																																								
11.	−	+	−	−	+	+	−	n	−	−	n	n	n	n	n	+	+	n	n	+	−	+	+	n	+	−	w	n	−	−	+	+	−	−	−	n	n	w	n	+
12.	+	w	−	−	−	−	−	n	+	−	n	n	n	n	n	v	+	n	n	+	w	+	+	n	−	−	−	n	−	−	+	+	−	−	−	n	n	−	n	+
13.	−	+	−	−	w	−	w	n	−	−	n	n	n	n	n	+	+	n	n	+	−	+	+	n	−	−	−	n	−	−	+	+	n	−	−	n	n	−	n	+
14.	w	−	−	−	−	−	w	n	−	−	n	n	n	n	n	w	w	n	n	+	−	+	+	n	−	−	−	n	−	−	+	+	n	−	−	n	n	−	n	+
Aureobasidium																																								
15.	v	v	+	v	×	×	+	n	+	n	+	+	+	×	n	n	n	n	n	n	n	n	n	n	+	n	n	+	n	n	+	+	n	−	−	10(H_2)	53.9	−	n	+
Babjevia																																								
16.	−	v	−	−	−	−	−	−	−	n	−	n	−	−	n	n	n	n	n	n	n	n	n	n	−	v	n	n	+	+	+	+	−	−	−	9	43.8	−	−	−
Bensingtonia																																								
17.	−	−	−	w	+	+	−	n	+	−	−	−	n	n	+	−	−	+	−	+	−	+	−	−	n	−	+	n	−	−	+	+	−	−	−	9	45.0	+	−	v
18.	−	−	+	+	+	+	−	n	+	+	−	+	n	n	+	+	+	+	−	+	−	+	+	−	n	−	+	n	−	−	+	+	−	−	−	9	55.7	+	−	−
19.	−	−	+	−	+	+	−	n	−	−	v	+	n	n	+	−	+	+	−	+	−	−	+	−	n	−	+	n	v	−	+	+	−	−	−	9	58.9	+	−	−
20.	−	−	−	w	+	−	−	n	+	+	−	−	n	n	+	−	+	+	−	−	−	−	−	−	n	−	+	n	−	−	+	+	−	−	−	9	47.4	+	−	−
21.	−	−	+	+	+	−	−	n	+	+	−	−	n	n	+	−	+	+	−	+	−	+	+	−	n	−	+	n	−	−	+	+	−	−	−	9	55.8	+	−	−
22.	+	+	w	−	+	−	−	n	+	+	−	−	n	n	+	−	+	+	−	−	−	+	−	−	n	−	+	n	−	−	+	+	−	−	−	9	50.4	+	−	+
23.	−	+	−	−	+	−	−	n	−	−	−	−	n	n	+	−	+	+	−	−	−	−	−	−	n	−	+	n	−	−	+	+	−	−	−	9	46.5	+	−	−
24.	−	−	+	−	v	v	−	n	−	−	−	+	n	n	v	−	−	+	−	−	−	+	+	−	n	−	+	n	−	−	+	+	−	−	−	9	53.7	+	−	+
25.	−	+	+	−	+	+	−	n	+	+	−	−	n	n	−	−	+	−	−	−	−	+	−	−	n	−	+	n	−	−	+	+	−	−	−	9	45.9	+	−	−
Blastobotrys																																								
26.	v	−	n	v	+	−	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	n	n	−	n	+
27.	v	+	n	−	+	v	v	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	n	n	−	n	+
28.	−	+	n	−	+	v	+	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	n	n	n	−	n	+
29.	−	v	n	−	+	v	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	n	n	−	n	+
30.	v	+	n	−	+	+	+	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	n	n	n	−	n	+
31.	+	+	n	v	+	+	+	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	n	n	n	−	n	+
Botryozyma																																								
32.	−	−	−	+	+	−	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	+	+	+	+	+	+	n	n	29.6	−	−	−
Brettanomyces																																								
33.	−	−	−	+	+	−	−	n	−	−	−	−	−	n	−	−	−	−	−	v	−	v	v	−	n	−	−	n	+	+	+	+	+	+	−	9	39.1	−	v	−
34.	−	v	−	v	+	−	−	n	−	−	−	v	−	n	v	−	−	−	−	+	−	+	+	−	n	−	−	n	+	+	+	+	−	−	−	9	42.7	−	−	−
35.	−	+	−	−	−	−	−	n	−	−	−	−	n	n	−	−	+	−	−	+	−	v	−	−	n	−	−	n	+	−	+	+	−	−	−	9	40.8	−	−	−
Bullera																																								
36.	−	w	+	+	+	−	+	n	−	+	−	+	n	n	+	+	+	−	−	−	−	+	−	−	n	+	+	n	−	−	+	+	n	−	−	10	55.5	+	+	−
37.	+	+	+	+	+	+	−	n	−	+	−	+	n	n	+	+	+	−	−	−	−	+	−	−	n	+	+	n	v	v	+	+	−	−	−	10	54.4	+	+	+
38.	+	+	+	+	+	+	+	n	−	−	−	+	n	n	+	+	−	v	−	−	−	+	+	−	n	+	+	n	v	−	+	+	v	−	−	10	38.5	+	+	−
39.	−	v	+	+	+	+	+	n	−	+	−	+	n	n	+	+	v	−	−	−	−	+	−	−	n	+	+	n	−	−	+	+	n	−	−	10	51.2	+	+	−
40.	−	+	v	−	+	+	−	n	+	+	−	+	n	n	v	−	+	v	−	−	−	−	−	−	n	v	+	n	−	−	+	+	−	−	−	10	49.8	+	−	+

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Bullera (*cont'd*)																																			
41. *B. miyagiana*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	−	w	+	w	+	+	+	−	n	−	+	−	+	+	−	+	+
42. *B. oryzae*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	n	−	−	−	+	×	+	+	+
43. *B. pyricola*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	n	−	+	+	−	+	+	+	+
44. *B. pseudoalba*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	n	−	+	+	−	+	+	+	+
45. *B. punicea*	−	−	−	−	−	−	−	+	−	v	+	+	+	+	−	−	+	+	−	+	−	+	+	+	v	−	n	−	v	+	−	+	v	+	+
46. *B. sinensis*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	+	+	+	−	v	+	+	+	+	+	+	n	−	+	+	+	+	+	+	+
47. *B. variabilis*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	v	+	+	+	−	+	+	+	+	+	+	+	n	−	−	v	v	v	+	+	+
Bulleromyces																																			
48. *B. albus*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	v	+	+	+	v	+	+	+	+	+	v	n	−	v	+	v	v	v	+	+
Candida																																			
49. *C. aaseri*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+	−	+	−	−	+	−	+	+	+	+	−	+	+
50. *C. albicans*	+	v	v	+	−	−	v	+	+	v	v	+	−	+	−	−	−	v	−	+	+	v	v	v	−	v	+	−	+	v	−	v	−	+	+
51. *C. amapae*	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	v	n	−	−	−	−	−	−	−	−
52. *C. anatomiae*	+	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	+	−	n	−	+	−	−	−	−	+	−
53. *C. ancudensis*	−	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+	+	−	−	−	+	+
54. *C. antillancae*	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+	+	−	−	−	+	+
55. *C. apicola*	+	−	+	−	−	v	−	+	−	+	+	−	−	−	−	−	+	−	−	−	v	−	−	v	−	−	+	−	v	+	−	v	−	+	+
56. *C. apis*	−	−	−	−	−	−	−	+	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+	+
57. *C. atlantica*	v	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+	−	−	+	+	+	+	+	+	n	−	+	+	+	+	−	+	+
58. *C. atmosphaerica*	+	v	v	v	−	−	+	+	+	+	+	+	+	+	−	−	−	+	−	−	+	+	+	+	−	+	+	−	+	+	+	+	−	+	+
59. *C. auringiensis*	+	+	−	−	+	−	+	+	+	+	−	−	+	+	+	−	−	−	−	−	+	+	+	v	−	+	n	−	+	+	+	+	+	+	+
60. *C. austromarina*	v	−	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	n	−	v	+	−	−	−	−	−
61. *C. azyma*	−	−	−	−	−	−	−	+	+	+	+	+	−	+	−	−	−	+	−	−	+	v	v	−	−	−	−	−	+	+	−	+	+	+	+
62. *C. beechii*	+	−	−	−	−	−	−	+	−	+	−	−	+	+	−	−	−	−	−	−	+	−	−	+	−	+	n	−	+	+	−	+	−	+	+
63. *C. bertae*	−	−	−	−	−	−	−	+	+	+	+	v	+	v	+	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+
64. *C. berthetii*	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	n	−	+	+	−	−	−	v	v
65. *C. blankii*	v	v	+	v	−	−	−	+	+	+	+	+	+	+	+	−	v	+	−	+	+	+	v	+	+	v	+	−	+	+	+	+	v	+	+
66. *C. boidinii*	+	−	−	−	−	−	−	+	−	v	−	−	−	−	−	−	−	−	−	−	+	v	v	v	v	v	+	+	+	+	+	+	−	+	+
67. *C. boleticola*	+	−	−	−	−	−	+	+	+	v	−	−	v	+	−	−	−	−	−	−	+	−	v	v	−	v	+	−	+	+	+	+	−	+	+
68. *C. bombi*	+	−	+	−	−	+	+	+	−	+	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+
69. *C. bombicola*	+	−	+	−	−	−	−	+	+	+	+	−	−	−	−	−	v	−	−	−	−	−	−	v	−	−	−	−	+	+	−	−	−	+	+
70. *C. buinensis*	+	+	−	v	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	+	v	+	v	−	+	n	−	+	+	−	−	−	+	v
71. *C. butyri*	+	v	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+	−	−	+	+	+	+	−	v	+	−	+	+	+	+	−	+	+
72. *C. cantarellii*	+	−	−	−	−	−	−	+	v	+	−	−	−	+	−	−	−	−	−	−	v	−	−	+	−	v	+	−	+	+	+	v	−	+	+
73. *C. caseinolytica*	−	−	−	−	−	−	−	+	+	v	v	−	−	+	−	−	−	−	−	−	v	−	−	−	−	−	−	−	+	+	−	−	−	−	−
74. *C. castellii*	+	−	−	−	−	−	−	+	−	−	−	−	−	v	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	+	−	−	−	−	−
75. *C. castrensis*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+	−	−	+	−	+	−	−	−	+	−	+	−	−	−	−	−	−
76. *C. catenulata*	v	v	−	v	−	−	−	+	+	−	−	v	−	+	−	−	−	−	−	v	v	−	−	v	−	v	−	−	+	+	−	+	−	+	+
77. *C. chilensis*	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+	+	+	−	v	+	−	+	+	+	+	−	+	+
78. *C. chiropterorum*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+
79. *C. coipomoensis*	+	+	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	v	−	−	+	−	+	+	−	+	n	−	+	+	+	+	−	+	+
80. *C. conglobata*	+	+	−	−	−	−	+	+	+	+	−	−	+	+	−	−	−	−	−	−	+	+	+	+	−	v	+	−	+	+	+	+	−	+	+
81. *C. cylindracea*	+	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+	+
82. *C. dendrica*	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−
83. *C. dendronema*	+	+	v	v	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+
84. *C. diddensiae*	+	+	v	v	−	−	+	+	+	v	+	+	+	+	−	−	−	+	−	−	+	+	+	+	−	+	n	−	+	+	+	+	−	+	+
85. *C. diversa*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	−	−	+	v	−	v	−	+	+
86. *C. drimydis*	−	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	+	−	+	+
87. *C. edax*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	v	+	+	+	+	+	v	n	−	+	+	+	+	+	+	+
88. *C. entomophila*	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	−	+	+	−	+	+	+	+	−	+	+
89. *C. ergastensis*	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	v	−	+	n	−	+	+	+	+	−	+	+

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Bullera (*cont'd*)																																								
41.	+	+	+	+	+	+	+	n	−	+	−	+	n	n	+	−	w	+	−	+	−	−	−	−	n	+	+	n	w	−	+	+	n	−	−	10	60.1	+	−	+
42.	+	+	+	+	+	+	+	n	−	+	−	+	n	n	+	+	+	−	−	+	−	+	+	−	n	+	+	n	−	−	+	+	n	−	−	10	61.2	+	+	−
43.	−	+	v	−	+	+	+	n	+	+	−	+	n	n	v	+	+	v	−	v	v	+	−	−	n	−	+	n	v	−	+	+	−	−	−	10	51.5	+	−	−
44.	+	+	+	+	+	+	+	n	−	−	−	+	n	n	+	+	+	−	−	+	−	+	+	−	n	−	+	n	+	−	+	+	n	−	−	10	53.1	+	−	+
45.	−	+	+	−	+	v	v	n	+	+	−	+	n	n	+	v	+	−	−	−	−	−	−	−	n	+	+	n	v	−	+	−	−	−	−	10	54.2	+	−	+
46.	+	+	+	+	+	+	+	n	−	−	−	+	n	n	+	+	+	v	−	+	−	+	+	−	n	+	+	n	+	−	+	+	n	−	−	10	55.1	+	+	−
47.	+	+	+	+	+	+	+	n	−	−	−	+	n	n	+	v	+	−	−	−	−	+	−	−	n	+	+	n	+	v	+	+	−	−	−	10	56.4	+	+	v
Bulleromyces																																								
48.	+	+	+	+	+	+	+	n	−	−	−	+	n	n	+	+	+	−	−	v	−	+	v	−	n	+	+	n	v	−	+	+	v	−	−	10	54.4	+	+	v
Candida																																								
49.	+	+	+	−	+	+	−	−	−	−	−	−	n	n	+	+	+	−	−	+	−	+	+	−	n	−	−	n	−	−	+	+	+	+	−	9	37.1	−	v	−
50.	v	−	v	+	+	+	−	+	−	−	v	+	n	n	+	v	−	v	v	+	−	+	+	v	v	−	−	n	+	+	+	+	+	+	+	9	35.0	−	−	+
51.	−	n	n	+	v	v	−	n	−	−	+	−	n	n	n	n	n	n	n	−	n	−	−	−	n	−	−	n	n	n	+	+	−	−	−	n	38.8	−	−	+
52.	−	+	+	−	+	v	−	n	−	+	−	+	n	−	−	−	+	+	+	+	−	+	+	−	n	−	−	n	+	−	+	+	−	−	−	9	39.0	−	−	−
53.	+	+	−	+	+	+	−	+	−	−	−	−	n	−	−	−	+	+	+	+	−	+	+	−	+	−	−	n	+	+	+	+	−	−	−	n	42.0	−	−	−
54.	−	+	−	+	v	−	−	−	−	−	−	−	n	−	−	−	+	+	+	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	39.0	−	−	−
55.	−	−	v	−	v	v	−	−	−	−	−	v	n	n	v	v	−	−	−	+	−	+	+	+	n	−	−	n	−	−	+	+	v	−	−	9	46.3	−	−	−
56.	−	−	+	−	−	+	−	−	−	−	−	−	−	n	−	−	−	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	w	−	−	9	47.2	−	−	−
57.	+	+	+	−	+	+	−	n	−	−	−	v	n	n	+	+	+	−	−	+	−	+	+	−	n	−	−	n	−	−	+	+	−	−	−	n	35.8	−	−	−
58.	+	+	+	−	+	+	−	+	−	−	−	v	n	−	+	+	+	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	−	−	−	9	39.0	−	−	−
59.	−	+	+	−	+	−	+	n	−	−	−	+	n	n	+	+	+	−	−	+	−	+	+	+	n	−	−	n	+	+	+	+	+	+	−	n	40.1	−	−	−
60.	−	−	v	−	+	−	−	n	−	−	−	v	n	n	−	−	−	−	−	−	−	−	−	−	−	−	−	n	−	−	n	−	−	−	−	n	39.0	−	−	−
61.	+	−	v	−	+	−	−	+	−	−	−	+	n	−	+	+	−	−	−	+	−	+	+	+	n	−	−	n	+	+	+	+	v	−	−	n	54.4	−	−	−
62.	−	+	+	−	+	+	−	n	−	−	−	+	n	n	+	−	+	−	−	+	−	+	+	+	n	−	−	n	+	+	+	+	−	−	−	n	40.9	−	−	−
63.	+	+	+	v	+	+	+	+	+	+	+	+	n	−	+	+	+	v	v	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	43.9	−	+	+
64.	−	+	−	+	+	+	−	n	+	+	+	v	n	n	−	−	+	+	v	+	−	+	+	−	n	−	−	n	+	+	+	+	+	+	+	n	39.8	−	+	−
65.	+	+	+	v	+	+	+	+	−	−	+	+	n	n	+	+	+	−	−	+	−	+	+	v	+	−	−	n	+	+	+	+	+	+	+	n	55.2	−	−	v
66.	−	−	−	+	+	−	−	−	v	+	−	v	−	n	+	+	−	v	−	+	−	+	+	−	−	−	−	n	+	+	+	+	v	v	−	7	32.4	−	+	v
67.	−	+	v	−	+	+	−	v	−	−	−	+	+	n	+	−	+	−	−	+	−	+	+	v	v	−	−	n	+	+	+	+	v	−	−	n	43.9	−	+	−
68.	−	−	+	−	+	+	−	−	−	−	−	−	n	n	−	−	−	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	+	+	−	9	47.5	−	−	−
69.	−	−	+	−	+	v	−	−	−	−	−	−	n	−	−	−	−	−	−	+	−	+	+	+	+	−	−	n	+	−	+	+	v	−	−	9	49.8	−	−	−
70.	+	+	+	−	+	+	−	n	−	−	−	−	−	n	+	+	+	−	−	+	−	+	+	−	n	−	−	n	−	−	+	+	−	−	−	n	31.5	−	−	−
71.	v	+	v	−	+	+	−	+	−	−	−	−	n	n	+	+	+	−	−	+	−	+	+	v	+	−	−	n	−	−	+	+	+	+	−	n	34.7	−	−	−
72.	−	−	v	v	+	v	−	−	−	−	+	+	n	n	+	−	−	−	−	−	−	+	+	v	+	−	−	n	+	+	+	+	v	−	−	9	41.9	−	−	−
73.	−	−	v	+	+	−	−	−	−	−	−	+	+	n	n	n	n	n	n	+	−	+	+	−	n	−	−	−	+	+	+	+	+	+	+	n	46.9	−	−	−
74.	−	−	+	−	−	−	−	−	−	−	−	+	−	n	−	−	−	−	−	−	−	+	−	+	+	−	−	n	−	−	+	+	+	+	−	n	43.3	−	−	−
75.	+	−	+	+	+	+	+	−	−	−	−	−	n	n	−	−	v	−	−	+	−	+	+	−	−	−	−	n	+	+	+	w	−	−	−	n	41.7	−	−	+
76.	−	−	v	+	+	+	−	−	−	−	−	v	n	n	v	−	−	v	+	+	−	+	v	+	n	−	−	n	+	+	+	+	+	+	−	9	53.8	−	−	−
77.	+	+	−	+	+	+	−	−	+	+	−	−	n	n	+	+	+	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	44.0	−	+	+
78.	+	+	+	+	−	+	+	−	−	−	−	+	n	−	−	−	+	−	−	+	−	+	+	+	+	−	−	n	+	+	+	+	+	+	−	n	48.0	−	−	+
79.	+	+	−	−	+	+	−	n	−	−	−	−	n	−	+	−	+	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	32.0	−	−	−
80.	−	+	+	−	+	−	−	+	−	−	−	−	−	n	+	+	+	−	+	+	−	+	+	+	n	−	−	n	−	−	+	+	v	−	−	n	40.1	−	−	−
81.	−	−	+	+	+	+	−	−	−	−	−	+	n	−	−	−	−	+	+	+	−	−	+	−	−	−	−	n	+	+	+	+	−	−	−	n	62.7	−	+	−
82.	−	+	+	+	+	+	−	−	−	−	−	−	−	n	−	−	+	−	−	−	−	+	+	−	n	−	−	n	+	+	+	+	−	−	−	n	42.0	−	−	−
83.	+	+	+	−	+	+	−	+	−	−	−	−	n	n	+	+	+	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	v	−	−	n	38.5	−	−	−
84.	+	+	+	−	+	+	−	n	−	−	−	−	n	n	+	+	+	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	+	+	v	9	37.7	−	+	−
85.	−	−	−	−	+	+	−	−	−	−	−	−	−	n	−	−	−	−	−	+	−	+	+	−	n	−	−	n	−	−	+	+	+	v	−	7	35.1	−	−	−
86.	+	+	−	−	+	+	−	−	−	−	−	−	n	−	−	−	+	+	+	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	43.4	−	−	−
87.	+	+	+	−	v	v	+	−	+	+	+	+	n	−	+	+	+	+	+	+	−	+	+	−	−	−	−	n	+	+	+	+	+	+	+	n	46.8	−	+	+
88.	+	+	v	−	+	+	−	−	−	−	−	v	n	−	+	+	+	+	+	+	−	+	+	v	+	−	−	n	+	+	+	+	+	+	v	n	56.3	−	−	+
89.	+	+	−	−	+	+	−	+	−	−	−	−	n	−	+	−	+	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	36.5	−	−	v

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

	Fermentation							Assimilation reactions and other characteristics																											
Species	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Candida (*cont'd*)																																			
90. *C. ernobii*	+	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	–	–	+	+	+	+	+	+	–	–	–	+	+	–	+	–	+	+
91. *C. etchellsii*	v	–	–	v	–	–	–	+	v	v	–	v	–	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	v	v	–	–	–	v	v
92. *C. ethanolica*	v	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	n	–	+	v	–	–	–	–	–
93. *C. fennica*	+	+	+	+	v	v	+	+	+	v	+	+	+	+	v	v	v	v	–	+	+	v	–	+	–	v	+	–	+	+	+	+	–	+	+
94. *C. fermenticarens*	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	+	+	+	+	+	+
95. *C. floricola*	+	–	+	+	–	+	–	+	+	+	+	+	–	–	–	–	+	–	–	–	v	–	–	+	–	–	–	–	+	+	–	–	–	+	+
96. *C. fluviatilis*	+	–	–	v	–	–	+	+	+	–	+	+	+	+	+	–	–	+	–	+	+	–	–	–	–	+	+	–	+	+	–	+	–	+	+
97. *C. freyschussii*	+	–	v	–	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	–	+	–	–	–	+	–	–	–	+	+	–	–	–	+	+
98. *C. friedrichii*	+	v	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	+	v	+	–	v	+	–	+	+	+	+	+	+	+
99. *C. fructus*	+	–	–	–	–	–	+	+	–	+	–	–	–	+	–	–	–	–	–	–	+	–	–	+	–	+	+	–	+	+	–	+	–	+	+
100. *C. galacta*	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	n	–	–	+	–	–	–	+	+
101. *C. geochares*	+	–	+	–	–	–	–	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	+	–	+	+
102. *C. glabrata*	+	–	–	–	–	–	v	+	–	–	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–	–	–	v	+	–	–	–	–	–
103. *C. glaebosa*	–	–	–	–	–	–	–	+	+	–	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	v	+	–	+	+	–	+	–	+	+
104. *C. glucosophila*	+	–	–	–	–	–	n	+	–	–	–	–	–	–	–	–	–	–	–	–	w	–	–	–	–	n	n	n	n	w	–	–	–	–	–
105. *C. gropengiesseri*	+	–	+	–	–	–	–	+	+	+	+	–	v	–	–	–	+	–	–	–	v	–	v	v	–	–	–	–	v	+	–	–	–	+	+
106. *C. haemulonii*	+	–	+	–	–	–	+	+	+	–	+	+	–	+	–	–	+	+	–	v	v	v	v	v	+	+	+	–	+	+	–	+	v	+	+
107. *C. homilentoma*	+	+	–	+	–	–	+	+	+	–	+	+	+	+	–	–	–	+	–	+	+	+	–	+	+	+	–	–	+	+	+	+	–	+	+
108. *C. humilis*	+	+	–	–	–	–	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	n	–	v	+	–	–	–	–	–
109. *C. incommunis*	+	–	v	–	–	–	–	+	v	+	+	+	+	+	–	–	–	+	–	–	v	–	–	–	–	+	+	–	+	+	+	–	–	+	+
110. *C. inconspicua*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–	–	–	–
111. *C. insectalens*	–	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	v	–	–	+	+	–	+	–	–	+	–	+	+
112. *C. insectamans*	+	–	–	–	–	–	–	+	–	–	–	+	+	+	–	–	–	+	–	+	+	–	–	+	–	–	–	–	+	v	–	+	–	+	+
113. *C. insectorum*	+	+	v	–	–	–	+	+	+	+	+	+	+	+	v	v	+	+	–	–	+	+	+	+	+	v	+	–	+	+	+	+	–	+	+
114. *C. intermedia*	+	+	+	v	–	v	v	+	+	+	+	+	+	+	+	–	+	+	v	v	+	v	v	v	v	v	n	–	+	v	–	+	v	+	+
115. *C. ishiwadae*	+	v	v	+	–	–	+	+	v	+	+	+	+	+	–	–	–	+	–	+	+	+	+	+	+	+	n	–	+	+	+	+	v	+	+
116. *C. karawaiewii*	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	v	–	+	v
117. *C. krissii*	–	–	–	–	–	–	–	+	–	+	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–	–	+	+
118. *C. kruisii*	+	+	–	–	–	–	+	+	+	+	+	+	+	+	–	–	–	+	–	+	+	+	–	v	–	+	+	–	+	+	–	+	–	+	+
119. *C. lactis-condensi*	+	–	+	–	–	+	–	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	n	–	–	–	–	–	–	–	–
120. *C. laureliae*	+	–	–	–	–	–	+	+	+	–	–	–	–	+	–	–	–	–	–	–	+	–	–	+	–	–	+	–	+	+	+	+	–	+	+
121. *C. llanquihuensis*	+	–	–	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	+	–	–	–	–	+	+	+	+	–	+	+
122. *C. lyxosophila*	+	+	–	+	–	–	–	+	+	–	+	+	+	+	–	–	–	+	–	+	+	–	–	+	–	+	+	–	+	+	–	+	–	+	+
123. *C. magnoliae*	+	–	+	–	–	–	–	+	+	+	v	–	v	v	–	–	v	–	–	–	v	–	–	v	–	–	–	–	v	+	–	v	–	+	+
124. *C. maltosa*	+	v	+	v	–	–	+	+	+	+	+	+	+	+	–	–	–	+	–	–	+	–	–	–	–	v	+	–	+	+	–	+	–	+	+
125. *C. maris*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	n	+	+	+	–	+	–	+	+
126. *C. maritima*	+	–	v	–	–	v	–	+	–	–	+	+	+	+	–	–	v	+	–	v	+	v	v	–	+	v	–	–	+	+	–	–	–	+	+
127. *C. melibiosica*	+	+	v	v	–	v	v	+	+	v	+	+	+	+	–	+	+	+	–	–	+	–	–	v	–	v	+	–	+	+	–	+	–	+	+
128. *C. membranifaciens*	+	v	+	v	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	v	+	+	+	+	v	v	+	–	+	+	+	+	+	+	+
129. *C. mesenterica*	v	–	–	–	–	–	–	+	–	+	+	+	+	+	–	–	–	v	–	–	v	–	v	v	–	v	n	–	+	+	+	+	–	+	+
130. *C. methanosorbosa*	+	+	–	–	–	–	+	+	+	+	–	–	v	+	–	–	–	–	–	–	+	+	–	+	+	+	+	+	+	+	–	+	–	+	+
131. *C. milleri*	+	+	+	–	–	+	+	+	+	–	+	–	–	+	–	–	+	–	v	–	–	–	–	–	–	–	–	–	v	v	–	–	–	–	–
132. *C. mogii*	+	–	+	v	–	–	+	+	+	–	+	+	–	+	–	–	+	–	v	v	+	+	v	+	–	+	+	–	+	+	–	v	v	+	+
133. *C. montana*	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–	+	–
134. *C. multigemmis*	+	–	–	–	–	–	v	+	+	+	+	+	–	+	–	–	–	+	–	–	+	+	v	v	–	v	n	–	–	–	–	+	–	–	+
135. *C. musae*	+	–	–	–	–	–	+	+	–	+	+	+	–	+	–	–	–	+	–	–	+	–	–	v	–	+	+	–	+	+	–	+	–	+	+
136. *C. naeodendra*	+	+	–	–	–	–	+	+	+	+	+	+	+	+	–	–	–	+	–	+	+	+	+	+	+	+	n	–	+	+	+	+	–	+	+
137. *C. nanaspora*	+	+	–	–	–	–	+	+	+	–	–	–	–	+	–	–	–	–	–	–	+	+	v	+	+	+	n	+	+	+	+	+	–	+	+
138. *C. natalensis*	+	+	v	–	–	–	–	+	+	+	+	+	+	+	–	–	–	+	–	–	+	–	–	+	–	–	+	–	+	+	–	+	–	+	+
139. *C. nemodendra*	–	–	–	–	–	–	–	+	+	+	–	–	v	+	–	–	–	–	–	–	+	+	+	+	–	–	n	+	+	+	+	+	+	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

	Assimilation reactions and other characteristics																																						
	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Candida *(cont'd)*																																							
90.	+	+	−	−	+	+	−	−	+	+	−	−	n	n	+	+	+	v	v	+	−	+	+	−	n	−	−	n	+	v	+	+	−	−	−	8	36.1	−	−	−
91.	−	−	v	−	v	v	−	−	+	+	−	v	−	n	v	−	−	−	−	v	−	+	+	+	+	−	−	n	−	−	+	+	−	−	−	9	52.6	−	−	−
92.	−	−	−	+	+	−	−	n	−	−	+	−	−	n	−	−	−	−	−	+	−	+	+	−	n	−	−	n	−	−	+	+	+	+	+	n	29.3	−	+	−
93.	+	+	+	−	+	+	−	v	−	−	+	+	n	n	+	v	+	−	v	+	−	+	+	+	+	−	−	n	−	−	+	+	v	−	−	n	36.3	−	−	+
94.	−	−	+	−	+	+	−	−	−	−	−	−	−	n	+	+	−	−	−	+	−	+	+	+	+	−	−	n	+	+	+	+	−	−	−	n	n	−	−	−
95.	−	−	v	−	+	−	−	−	−	−	−	−	n	n	v	−	−	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	+	−	−	9	51.7	−	−	−
96.	+	+	+	+	+	+	−	+	−	−	−	+	−	n	+	−	+	+	+	+	−	+	+	−	−	−	−	n	+	+	+	+	+	+	−	n	39.9	−	−	−
97.	+	+	+	+	+	+	−	−	−	−	−	−	n	n	+	−	+	+	+	+	−	+	+	−	−	−	−	n	−	−	+	+	+	+	+	n	44.3	−	−	−
98.	+	+	+	v	+	+	−	+	−	−	−	+	n	n	+	+	+	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	v	−	−	n	33.3	−	+	−
99.	−	−	+	−	+	+	−	−	−	−	−	+	n	−	+	−	+	+	−	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	n	48.8	−	−	−
100.	−	−	+	−	+	+	−	n	−	−	−	−	n	−	−	−	−	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	+	−	−	8	50.0	−	−	−
101.	−	+	+	−	+	+	−	−	−	−	−	+	−	n	+	−	+	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	+	w	−	n	54.1	−	−	−
102.	−	−	+	v	−	−	−	−	−	−	−	v	−	n	−	−	−	−	−	+	−	v	−	v	+	−	−	n	−	−	+	+	+	+	+	6	39.9	−	−	−
103.	+	+	−	+	+	+	−	v	−	−	−	+	−	n	+	−	+	+	−	+	−	+	+	−	−	−	−	n	+	−	+	+	−	−	−	9	42.7	−	−	−
104.	−	−	−	−	−	−	−	n	+	n	−	n	n	n	n	n	n	n	n	n	n	−	+	+	n	n	−	n	−	−	n	+	+	+	n	9	36.6	−	−	−
105.	−	+	+	−	+	+	−	+	−	v	−	+	n	n	+	−	+	−	−	+	−	+	+	+	n	−	−	n	v	−	+	+	v	v	−	9	56.4	−	−	−
106.	−	−	+	−	+	+	−	+	−	−	−	+	−	−	v	v	v	v	−	+	−	+	−	+	+	−	−	n	+	+	+	+	+	+	−	9	46.0	−	−	−
107.	+	+	v	−	+	+	−	−	−	−	v	+	n	n	+	+	+	−	−	+	−	+	+	+	+	−	−	n	+	+	+	+	+	+	−	n	48.7	−	−	+
108.	−	−	−	+	−	−	−	n	−	−	−	−	−	n	−	−	−	−	−	−	−	−	−	−	+	−	−	n	−	−	+	+	w	−	−	n	47.1	−	−	−
109.	+	+	+	−	+	+	+	+	+	+	−	+	+	−	+	−	+	+	−	+	−	+	+	−	n	−	−	n	+	+	+	+	+	v	−	8	48.9	−	−	−
110.	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	n	−	−	+	+	+	+	+	8	36.5	−	−	−
111.	−	+	−	+	+	−	−	−	−	−	v	−	n	−	−	−	v	−	−	+	−	+	+	−	−	−	−	n	−	−	+	+	+	+	+	n	44.9	−	−	−
112.	+	+	−	−	+	+	−	−	−	−	−	+	n	−	−	−	+	−	−	+	−	+	+	−	−	−	−	n	−	−	+	+	+	+	+	n	35.9	−	−	−
113.	+	+	+	−	+	+	−	+	−	−	−	−	n	−	+	+	+	−	−	+	−	+	+	−	n	−	−	n	v	v	+	+	+	+	−	n	39.5	−	−	+
114.	+	+	v	−	+	+	−	n	−	−	v	+	n	n	v	−	+	v	−	+	−	+	+	v	n	−	−	n	v	−	+	+	v	−	−	9	44.1	−	+	−
115.	+	+	+	+	+	+	−	n	+	+	−	+	n	−	+	+	+	v	v	+	−	+	+	+	+	−	−	n	+	+	+	+	+	−	−	n	36.7	−	−	−
116.	−	−	−	−	−	−	−	−	−	−	−	+	n	n	−	−	−	−	−	+	−	−	+	−	−	−	−	n	−	−	+	+	w	−	−	8	35.9	−	−	−
117.	−	+	+	−	+	+	−	−	−	−	−	+	+	n	−	−	v	−	−	+	−	+	+	+	+	−	−	n	+	+	+	+	−	−	−	n	43.4	−	−	−
118.	+	+	+	−	+	+	−	−	−	−	+	+	−	n	+	−	+	−	−	+	−	+	+	v	+	−	−	n	+	+	+	+	−	−	−	9	44.6	−	−	−
119.	−	−	−	−	−	−	−	n	+	+	−	−	−	n	−	−	n	−	−	−	−	v	−	+	n	−	−	n	v	−	+	+	v	−	−	8	42.8	−	−	−
120.	−	+	−	−	+	+	−	+	−	−	−	+	n	−	+	−	+	−	−	+	−	+	+	−	v	−	−	n	+	+	+	+	−	−	−	n	44.9	−	−	−
121.	−	−	−	+	+	+	−	−	−	−	−	−	n	−	+	+	−	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	40.5	−	−	−
122.	+	+	−	+	+	+	−	−	−	−	−	+	n	−	+	−	+	−	−	+	−	+	+	−	−	−	−	n	v	−	+	+	v	−	−	n	38.1	−	−	+
123.	−	v	+	−	+	+	−	−	+	+	−	+	−	n	v	−	v	v	v	+	−	+	+	+	+	−	−	n	v	−	n	+	+	+	−	9	60.0	−	−	−
124.	+	+	v	v	+	v	−	+	−	−	−	+	+	n	+	−	+	v	−	+	−	+	+	+	v	−	−	n	+	+	+	+	+	+	−	9	36.7	−	−	−
125.	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	+	+	−	−	−	−	n	+	−	+	+	+	w	−	7	49.1	−	−	−
126.	+	+	+	+	+	+	−	−	−	−	−	v	n	n	+	v	+	v	−	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	n	46.1	−	−	−
127.	v	+	+	−	+	+	−	+	−	−	v	+	−	n	+	−	+	−	−	+	−	+	+	v	+	−	−	n	−	−	+	+	+	+	v	9	55.0	−	−	−
128.	v	+	+	+	+	+	−	+	−	−	−	+	−	n	+	+	+	v	−	+	−	v	+	+	+	−	−	n	−	−	+	+	v	v	−	n	33.4	−	−	v
129.	+	+	−	−	+	+	−	n	−	−	−	+	−	n	v	−	+	−	−	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	n	47.6	−	+	+
130.	−	+	+	+	+	+	−	−	+	+	−	−	−	n	+	+	+	−	−	+	−	+	+	−	−	−	−	n	+	−	+	+	+	+	+	n	n	−	−	−
131.	−	−	−	v	v	−	−	−	−	−	−	−	−	n	−	−	−	−	−	−	−	−	−	−	−	−	−	n	v	v	+	+	v	−	−	n	47.3	−	−	−
132.	−	−	+	−	+	+	−	−	−	−	−	−	−	n	+	+	−	−	−	+	−	+	+	+	−	−	−	n	−	−	+	+	+	+	−	9	46.1	−	−	−
133.	−	+	−	+	+	+	−	−	−	−	−	−	n	n	−	−	+	+	+	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	7	35.6	−	−	−
134.	−	−	v	−	+	+	−	n	−	−	−	+	−	−	+	−	−	−	−	+	−	+	+	v	v	−	−	n	−	−	+	+	−	−	−	n	35.4	−	−	−
135.	+	−	+	−	+	+	−	−	−	−	−	+	−	−	+	−	+	−	−	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	n	47.9	−	−	−
136.	+	+	+	−	+	+	−	n	−	−	−	−	−	−	+	+	+	−	−	+	−	+	+	+	−	−	−	n	−	−	+	+	+	+	+	n	38.3	−	−	−
137.	−	−	−	−	+	−	−	n	+	+	−	−	n	−	+	+	−	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	+	+	−	7	36.6	−	−	−
138.	−	+	+	+	+	+	−	+	−	−	−	+	+	−	−	−	+	−	−	+	−	+	+	−	−	−	−	n	+	−	+	+	−	−	−	n	37.4	−	−	−
139.	−	+	−	v	+	−	−	n	−	−	v	−	−	n	+	+	+	−	−	+	−	+	+	−	−	−	−	n	+	v	+	+	w	−	−	7	41.8	−	−	−

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																												
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Candida (*cont'd*)																																			
140. *C. nitratophila*	+	v	−	−	−	−	+	+	+	−	−	−	−	+	−	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	−	+	−	+	+
141. *C. norvegica*	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−	v	−	v	−	−	−	+	+	−	−	−	+	+
142. *C. odintsovae*	+	−	+	−	−	+	−	+	−	−	+	+	+	+	−	−	+	+	v	−	+	+	−	v	+	−	−	−	+	+	−	+	−	+	+
143. *C. oleophila*	+	+	v	v	−	−	−	+	+	+	+	+	+	+	−	−	−	+	−	−	+	−	v	v	−	+	+	−	+	+	−	+	−	+	+
144. *C. oregonensis*	+	−	−	+	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	+	+	−	v	v	+	+	+	−	+	−	−	+	−	+	+
145. *C. ovalis*	+	−	−	−	−	−	+	+	−	+	−	−	+	+	−	−	−	−	−	−	+	+	+	+	−	−	n	+	+	+	+	+	−	+	+
146. *C. palmioleophila*	−	−	−	−	−	−	−	+	+	−	+	+	−	+	−	+	+	+	−	+	+	−	−	−	−	+	+	−	+	+	−	+	−	+	+
147. *C. paludigena*	v	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	v	+	−	−	+	+	−	−	−	+	+	−	+	−	−	−	−	v	−
148. *C. parapsilosis*	+	v	v	v	−	−	v	+	+	+	+	+	−	+	−	−	−	+	−	−	+	+	−	v	−	v	+	−	+	+	−	+	−	+	+
149. *C. pararugosa*	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	+	+	v	−	−	−	−	−	+	+	−	−	−	+	+
150. *C. peltata*	+	+	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+	−	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+
151. *C. petrohuensis*	−	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−	−	−	−	−	+	v	−	−	−	v	+	−	+	+	−	+	−	+	+
152. *C. pignaliae*	+	−	−	−	−	−	+	+	−	v	−	−	−	+	−	−	−	−	−	−	+	+	−	v	−	−	−	+	+	+	−	+	−	+	+
153. *C. pini*	+	−	−	−	−	−	v	+	−	−	−	−	−	+	−	−	−	−	−	−	+	+	−	+	−	−	−	+	+	+	+	−	−	+	+
154. *C. populi*	+	−	−	−	−	−	w	+	−	−	+	+	+	+	−	−	−	+	−	−	+	+	+	+	+	+	+	−	+	+	−	+	−	+	+
155. *C. pseudointermedia*	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	−	+	+	v	v	+	−	−	+	+	+	n	−	+	−	−	+	+	+	+
156. *C. pseudolambica*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	−	−	−	−	−
157. *C. psychrophila*	−	−	−	−	−	−	−	+	+	v	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−	−	n	−	+	+	+	+	−	+	+
158. *C. quercitrusa*	+	v	v	v	−	−	−	+	+	+	+	+	−	+	−	−	−	+	−	−	v	−	−	+	−	+	+	−	+	+	−	v	−	+	+
159. *C. quercuum*	w	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	+	+
160. *C. railenensis*	+	+	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	−	−	+	−	+	−	−	+	+	−	+	+	−	+	−	+	+
161. *C. rhagii*	+	v	+	−	−	v	+	+	+	v	+	+	+	+	−	−	+	+	−	−	+	v	−	+	v	+	+	−	+	+	+	+	−	+	+
162. *C. rugopelliculosa*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	n	−	+	−	−	−	−	−	−
163. *C. rugosa*	−	−	−	−	−	−	−	+	+	v	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	+	−	+	+	−	v	−	+	+
164. *C. saitoana*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	v	+	+	v	+	v	+	v	−	v	−	v	+	−	+	+	−	+	−	+	+
165. *C. sake*	+	+	v	v	−	−	v	+	+	+	+	+	v	+	−	−	−	+	−	−	+	−	−	v	−	v	v	−	+	+	−	v	−	+	+
166. *C. salmanticensis*	+	+	+	+	−	+	+	+	+	+	+	+	+	+	v	+	+	+	+	−	+	−	+	−	−	+	+	−	+	−	−	+	−	+	−
167. *C. santamariae*	+	−	−	−	−	−	+	+	v	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	+	−	+	+
168. *C. santjacobensis*	+	+	−	−	−	−	+	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	+	+	v	+	+	−	+	+	+	+	−	+	+
169. *C. savonica*	+	+	−	−	−	−	+	+	+	−	−	−	+	+	−	−	−	−	−	−	+	−	+	−	−	+	+	−	+	+	−	+	−	+	+
170. *C. schatavii*	+	+	−	−	−	−	+	+	+	−	−	−	−	+	−	−	−	−	−	−	+	−	v	+	−	+	+	−	+	+	+	+	−	+	+
171. *C. sequanensis*	+	+	−	−	−	−	−	+	+	−	−	+	+	+	−	−	−	−	−	+	+	+	−	+	−	−	+	−	+	+	+	+	−	+	+
C. shehatae																																			
172. var. *shehatae*	+	+	−	+	−	−	+	+	+	v	+	+	+	+	v	−	v	+	−	+	+	v	v	+	−	v	+	−	+	+	v	+	−	+	+
173. var. *insectosa*	+	+	−	+	−	−	+	+	+	−	+	+	+	+	v	−	v	+	−	+	+	−	v	+	−	v	+	−	+	+	−	+	−	+	+
174. var. *lignosa*	+	+	−	+	−	−	+	+	+	+	+	+	+	+	v	−	v	+	−	+	+	v	v	+	−	v	+	−	+	+	v	+	v	+	+
175. *C. silvae*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	+	+	−	+	−	+	+
176. *C. silvanorum*	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+
177. *C. silvatica*	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	n	−	+	+	−	+	−	+	+
178. *C. silvicultrix*	+	+	+	+	−	+	−	+	+	−	+	+	+	+	−	+	+	+	−	+	+	+	+	+	−	−	−	−	+	+	+	+	−	+	+
179. *C. solani*	+	−	−	−	−	−	−	+	−	v	+	+	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	+	v
180. *C. sonorensis*	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	v	−	−	−	+	+	+	−	+	−	+	+
181. *C. sophiae-reginae*	+	+	−	−	−	−	+	+	+	+	+	+	−	+	−	−	−	+	−	−	−	−	−	+	−	−	+	−	+	+	+	+	−	+	+
182. *C. sorbophila*	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	+	v	−	−	−	−	−	−	+	+	−	−	−	+	+
183. *C. sorboxylosa*	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−
184. *C. spandovensis*	+	+	+	−	−	v	−	+	+	+	+	−	−	−	−	−	+	−	−	−	+	+	−	−	−	−	−	−	+	+	−	+	+	+	+
185. *C. stellata*	+	−	+	−	−	+	−	+	−	v	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	n	−	−	−	−	−	−	−	−
186. *C. succiphila*	+	+	−	−	−	−	+	+	+	+	−	−	+	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	v	+	+
187. *C. suecica*	v	−	−	−	−	−	−	+	−	+	+	+	v	+	−	−	−	−	−	−	+	−	−	−	−	−	v	−	+	+	−	+	−	+	+
188. *C. tanzawaensis*	+	−	−	−	−	−	w	+	+	−	+	+	+	+	−	−	−	+	−	−	−	−	−	−	−	−	+	−	+	+	−	+	−	+	+
189. *C. tenuis*	v	v	v	v	−	−	v	+	+	v	+	+	+	+	+	−	v	+	−	v	+	v	+	+	+	v	+	−	+	+	v	+	−	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Candida (*cont'd*)																																								
140.	−	v	−	−	+	−	−	−	+	+	−	−	−	n	+	+	−	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	×	−	−	7	36.6	−	−	−
141.	−	+	v	+	+	+	−	−	+	+	−	−	−	n	v	−	+	+	+	+	−	+	+	−	−	−	−	n	v	v	+	+	−	−	−	n	41.2	−	−	−
142.	+	+	+	+	+	+	−	−	−	−	−	−	n	n	+	+	+	+	+	+	−	+	+	−	−	−	−	n	−	−	+	+	+	+	−	7	36.6	−	+	−
143.	v	+	+	v	+	+	−	v	−	−	−	+	n	n	+	−	+	−	−	+	−	+	+	+	v	−	−	n	+	+	+	+	−	−	−	9	41.2	−	+	−
144.	+	+	+	−	+	+	−	+	−	−	−	+	n	n	v	−	+	−	−	+	−	+	+	−	−	−	−	n	v	−	+	+	−	−	−	9	48.1	−	−	−
145.	−	+	+	+	+	+	−	n	−	−	−	−	n	−	+	+	+	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	+	−	−	7	35.8	−	−	−
146.	+	−	+	+	+	+	−	+	−	+	−	+	n	n	+	−	+	+	+	+	−	+	+	−	+	−	−	n	−	−	+	+	+	+	+	9	39.6	−	+	−
147.	+	+	+	−	+	+	+	−	−	−	−	+	+	−	−	−	+	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	w	−	−	n	41.5	−	−	+
148.	+	−	+	−	+	+	−	+	−	−	−	+	v	n	+	v	−	v	−	+	−	+	+	+	+	−	−	n	v	−	+	+	+	+	v	9	40.5	−	−	−
149.	−	−	−	+	+	−	−	−	−	−	−	−	−	n	+	−	−	+	+	+	−	+	+	−	−	−	−	n	−	−	+	+	+	+	−	n	48.3	−	+	−
150.	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	+	+	−	−	−	−	n	+	+	+	+	+	+	+	n	n	−	v	−
151.	+	v	−	+	+	−	−	−	−	−	−	−	n	−	−	−	+	+	+	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	46.1	−	−	−
152.	−	−	−	−	+	−	−	−	+	+	−	−	−	−	+	+	−	+	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	7	43.7	−	−	−
153.	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	−	−	+	−	+	+	−	−	−	−	n	+	−	+	+	−	−	−	7	37.3	−	−	−
154.	+	+	+	−	+	+	−	−	+	+	−	−	n	n	+	+	+	+	+	+	−	+	+	−	−	−	−	n	+	+	+	+	v	−	−	8	38.2	−	−	−
155.	+	+	+	−	+	+	−	+	−	−	−	+	n	n	+	−	+	−	−	+	−	+	+	+	+	−	−	n	+	−	+	+	+	−	−	n	44.9	−	−	−
156.	−	−	−	+	+	−	−	−	−	−	+	−	n	n	−	−	−	−	−	+	−	+	+	−	−	−	−	n	−	−	+	+	+	−	−	n	31.1	−	−	−
157.	−	−	−	−	−	−	−	n	−	−	−	+	n	n	+	+	−	−	−	−	−	−	−	+	n	−	−	n	−	−	−	−	−	−	−	n	36.1	−	−	−
158.	+	−	+	+	+	+	−	+	−	−	−	+	+	n	+	−	−	−	−	+	−	+	+	v	−	−	−	n	v	−	+	+	+	−	−	9	40.3	−	−	−
159.	+	+	+	+	+	+	−	−	−	−	−	−	n	n	+	−	+	+	+	+	−	+	+	−	−	−	−	n	−	−	+	+	+	−	−	n	38.9	−	−	−
160.	+	+	+	−	+	+	−	v	−	−	−	+	n	n	+	−	+	+	−	+	−	+	+	+	v	−	−	n	+	+	+	+	−	−	−	n	41.8	−	+	+
161.	+	+	+	v	+	−	−	+	−	−	+	+	n	n	+	+	+	−	−	+	−	+	+	v	+	−	−	n	−	−	+	+	v	−	−	n	42.4	−	−	−
162.	−	−	−	+	+	−	−	−	−	−	+	−	−	n	−	−	−	+	−	+	−	+	+	−	+	−	−	n	−	−	+	+	+	+	−	n	30.0	−	+	−
163.	−	−	v	+	v	v	−	+	−	−	−	−	−	−	v	−	−	+	+	+	−	+	+	+	n	−	−	n	−	−	+	+	+	+	v	9	50.5	−	+	−
164.	+	+	+	+	+	+	−	+	−	v	v	+	n	n	+	v	+	+	+	+	−	+	+	v	+	−	−	n	+	v	+	+	v	v	v	9	37.1	−	−	−
165.	v	v	v	v	+	v	−	v	−	−	v	+	v	n	v	−	v	−	v	+	−	+	v	v	v	−	−	n	−	−	+	+	−	−	−	9	39.4	−	−	−
166.	+	+	+	+	+	−	−	−	−	−	−	+	+	n	+	−	+	+	+	+	−	+	+	+	+	−	−	n	+	+	+	+	−	−	−	n	45.5	−	−	−
167.	−	+	+	v	+	+	−	−	−	−	−	+	+	−	+	−	+	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	9	37.2	−	+	−
168.	+	+	+	−	+	+	+	−	−	−	−	+	n	−	+	+	+	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	48.5	−	−	−
169.	−	+	−	−	+	+	−	−	−	−	+	+	−	n	+	−	+	−	−	+	−	+	+	+	+	−	−	n	+	+	+	+	−	−	−	n	48.0	−	−	−
170.	−	−	+	−	+	+	−	+	−	−	+	+	+	n	+	−	−	−	−	+	−	+	+	v	−	−	−	n	+	+	+	+	+	v	−	n	44.2	−	−	−
171.	−	+	+	−	+	+	−	+	−	−	−	+	n	−	+	+	+	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	−	−	−	n	39.6	−	−	−
172.	+	+	v	−	+	+	−	+	−	−	−	+	n	n	+	v	+	−	−	+	−	+	+	−	v	−	−	−	+	v	+	+	−	−	−	9	43.6	−	−	−
173.	+	+	v	−	+	+	−	+	−	−	−	+	n	n	v	−	+	−	−	+	−	+	+	−	n	−	−	w	+	+	+	+	−	−	−	9	44.5	−	−	−
174.	+	+	−	+	+	+	−	+	−	−	−	+	n	n	+	−	+	−	−	+	−	+	+	−	n	−	−	−	+	+	+	+	−	−	−	9	44.1	−	−	−
175.	−	−	v	v	+	v	−	−	−	−	−	−	n	n	−	−	−	v	−	+	−	+	+	−	−	−	−	n	−	−	+	+	+	v	−	7	39.5	−	−	−
176.	+	+	+	−	+	+	−	−	−	−	−	−	n	n	+	+	+	−	−	+	−	+	+	+	−	−	−	n	−	−	+	+	+	+	−	n	41.1	−	−	−
177.	−	−	−	+	+	−	−	n	−	−	−	−	n	−	−	−	v	−	−	+	−	+	+	−	n	−	−	n	−	−	+	+	+	+	−	n	55.6	−	−	−
178.	+	+	+	+	+	+	−	−	−	−	+	−	n	n	+	+	+	+	+	+	−	+	+	+	+	−	−	n	−	−	+	+	+	+	−	n	36.6	−	−	−
179.	+	+	+	+	+	−	−	−	−	−	−	+	−	−	+	−	+	+	+	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	7	41.9	−	−	−
180.	−	+	−	+	+	−	−	−	−	−	−	−	−	−	+	+	+	v	−	+	−	+	+	−	−	−	−	n	+	+	+	+	+	+	+	7	36.1	−	−	−
181.	+	−	−	−	+	+	−	−	−	−	−	+	n	−	+	−	−	−	−	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	n	39.3	−	−	−
182.	−	−	−	−	+	−	−	+	−	−	−	−	−	n	+	−	−	−	−	+	−	+	+	−	+	−	−	n	+	+	+	+	+	v	−	n	50.0	−	−	−
183.	−	−	−	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	+	+	v	+	−	−	n	−	−	+	+	+	v	−	n	40.3	−	−	−
184.	−	−	−	+	+	+	−	−	−	−	−	v	n	−	+	v	−	−	−	+	−	+	+	+	+	−	−	n	−	−	+	+	−	−	−	n	52.7	−	−	−
185.	−	−	−	−	−	−	−	n	−	−	−	−	−	n	−	−	−	−	−	−	−	+	−	+	n	−	−	n	−	−	+	+	v	−	−	8	42.0	−	−	−
186.	−	+	v	v	+	−	−	−	−	−	v	+	n	−	+	+	+	−	−	−	−	−	+	−	−	−	−	n	+	+	+	+	+	+	−	7	40.4	−	−	−
187.	+	v	−	−	−	+	−	−	−	−	−	+	n	−	−	−	v	−	−	−	−	−	−	−	−	−	−	n	−	−	+	+	−	−	−	n	42.6	−	+	−
188.	+	+	−	−	+	+	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	+	+	−	+	−	−	n	−	−	+	+	−	−	−	9	45.1	−	+	v
189.	+	+	+	v	+	+	−	v	−	−	−	+	n	−	+	v	+	v	−	+	−	+	+	v	−	−	−	n	v	v	+	+	−	−	−	9	43.7	−	−	v

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Candida (*cont'd*)																																			
190. *C. tepae*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+	+	−	−	−	+	+
191. *C. torresii*	+	−	−	−	−	−	+	+	+	+	−	−	+	+	−	−	−	−	−	−	+	−	−	v	−	+	+	−	+	+	−	+	−	+	+
192. *C. tropicalis*	+	+	v	+	−	−	+	+	+	v	v	+	+	+	−	−	−	v	−	+	+	−	−	v	−	v	+	−	+	v	−	+	−	+	+
193. *C. tsuchiyae*	+	−	+	+	−	+	+	+	−	+	+	+	−	+	−	−	+	+	−	+	−	−	−	−	−	+	−	−	+	+	−	+	−	+	+
194. *C. vaccinii*	+	−	+	−	−	v	−	+	+	+	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+
195. *C. valdiviana*	+	−	−	−	−	−	−	+	+	+	+	+	+	+	v	+	+	+	−	−	+	v	v	−	−	+	+	−	+	+	−	v	v	+	+
196. *C. vanderwaltii*	−	−	−	−	−	−	−	+	+	+	v	−	−	+	−	−	−	−	−	−	+	+	+	+	−	−	n	−	−	+	−	+	+	+	+
197. *C. vartiovaarae*	+	−	+	v	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	+	+
198. *C. versatilis*	+	+	v	v	v	v	v	+	+	−	v	v	+	+	v	v	v	−	−	−	v	v	−	v	−	−	+	−	+	+	−	v	−	+	−
199. *C. vinaria*	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	v	−	−	v	−	+	+	−	+	+	−	−	−	v	v
200. *C. vini*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	v	−	v	−	+	+
201. *C. viswanathii*	+	+	v	+	−	−	+	+	+	v	+	+	+	+	−	−	−	+	−	+	+	v	−	v	−	v	+	−	+	+	−	+	−	+	+
202. *C. wickerhamii*	+	−	−	−	−	−	v	+	+	−	−	−	+	v	−	−	−	−	−	−	+	+	+	+	+	v	+	−	+	+	−	+	−	+	+
203. *C. xestobii*	+	−	−	−	−	−	−	+	+	+	+	+	v	+	−	+	+	v	−	−	+	+	−	+	−	+	n	−	+	+	−	+	−	−	−
204. *C. zeylanoides*	v	−	−	−	−	−	v	+	v	+	−	−	v	+	−	−	−	−	−	−	−	−	−	−	−	v	+	−	v	+	−	v	−	+	+
Cephaloascus																																			
205. *C. albidus*	w	w	−	−	−	−	−	+	+	+	−	−	×	+	−	+	+	−	−	−	+	+	v	−	−	−	+	−	+	+	−	+	−	+	+
206. *C. fragrans*	−	−	−	−	−	−	−	+	+	v	−	−	−	v	−	−	−	−	−	v	+	+	−	−	−	−	v	−	+	+	−	+	−	+	+
Chionosphaera																																			
207. *C. apobasidialis*	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Citeromyces																																			
208. *C. matritensis*	+	−	×	v	−	w	v	+	−	+	+	+	−	+	−	−	+	−	v	−	−	−	v	−	−	v	+	−	×	×	−	v	−	+	+
Clavispora																																			
209. *C. lusitaniae*	+	v	v	v	−	−	v	+	+	+	+	+	+	+	−	−	−	+	−	−	+	v	−	−	v	−	+	−	+	+	−	+	−	+	+
210. *C. opuntiae*	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	−	−	+	−	−	+	−	−	−	−	v	+	−	+	+	−	+	−	+	+
Cryptococcus																																			
211. *C. aerius*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	w	−	−	+	+	−	+	+	+	+
212. *C. albidosimilis*	−	−	−	−	−	−	−	+	−	−	+	+	+	v	+	w	−	+	n	+	+	+	−	−	+	−	n	n	×	−	−	−	−	+	+
213. *C. albidus*	−	−	−	−	−	−	−	+	v	v	+	+	+	×	v	v	+	+	−	v	+	+	v	v	v	−	−	−	×	v	v	v	v	+	+
214. *C. amylolentus*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	v	+	+
215. *C. antarcticus*	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	v	+	n	+	+	v	−	−	+	−	n	n	−	−	−	−	−	+	v
216. *C. aquaticus*	w	−	v	v	−	v	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	−	−	−	+	v	−	+	−	+	+
217. *C. ater*	−	−	−	−	−	−	−	+	×	v	+	+	+	+	+	−	+	v	−	−	+	+	v	v	+	+	+	−	−	−	−	−	−	+	+
218. *C. bhutanensis*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	+	w	w	+	+	+	−	v	−	−	−	v	−	−	−	−	+	+
219. *C. consortionis*	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−	−	+	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	+	−
220. *C. curvatus*	−	−	−	−	−	−	−	+	+	−	+	×	+	+	+	−	+	v	−	+	+	v	−	+	×	+	+	−	×	+	×	×	−	v	v
221. *C. dimennae*	−	−	−	−	−	−	−	+	+	w	+	−	+	+	+	−	+	−	−	−	+	+	+	+	+	−	−	−	+	v	−	+	+	+	+
222. *C. feraegula*	−	−	−	−	−	−	−	+	−	+	−	−	+	+	−	−	−	−	−	−	+	+	+	+	+	−	−	−	+	+	−	−	−	+	+
223. *C. flavus*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	w	+	+	+	w	+	+	w	+	−	−	−	+	w	w	+	+
224. *C. friedmannii*	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−
225. *C. fuscescens*	−	−	−	−	−	−	−	+	v	−	−	−	+	+	−	−	−	−	−	−	+	+	−	+	−	−	w	−	−	−	−	−	−	+	−
226. *C. gastricus*	−	−	−	−	−	−	−	+	+	−	−	+	+	+	v	−	−	+	−	+	+	+	−	w	+	−	−	−	v	v	−	−	−	+	v
227. *C. gilvescens*	−	−	−	−	−	−	−	+	+	−	−	+	+	+	+	−	−	+	−	+	−	+	−	−	v	−	n	−	+	+	−	−	−	+	+
228. *C. heveanensis*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	+	+	−	−	+	+	+	+	+	−	−	−	+	−	+	+	+	+	+
229. *C. huempii*	−	−	−	−	−	−	−	+	+	+	−	+	+	+	+	−	−	+	−	+	+	+	+	w	+	+	+	−	+	−	−	+	+	+	+
230. *C. humicolus*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	v	+	v	v	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
231. *C. hungaricus*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	v	v	+	+	−	−	+	+	×	×	+	−	+	−	−	−	−	v	+	+	+
232. *C. kuetzingii*	−	−	−	−	−	−	−	+	−	−	+	−	+	+	−	−	+	−	−	−	+	+	w	w	−	−	−	−	+	v	−	w	−	+	+
233. *C. laurentii*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	+	+	+	−	v	+	+	+	+	+	−	−	−	v	v	v	+	+	+	+
234. *C. luteolus*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	−	+	+	+	−	−	+	+	v	×	+	w	+	−	v	v	+	v	+	+	×
235. *C. macerans*	−	−	−	−	−	−	−	+	×	v	+	+	+	+	v	−	+	×	−	v	+	+	+	v	v	−	−	−	v	v	+	−	−	+	+

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Candida (*cont'd*)																																								
190.	−	v	−	+	+	−	−	−	−	−	−	−	n	−	−	−	+	+	+	−	−	−	−	−	−	−	−	n	+	+	+	+	−	−	−	n	41.0	−	−	−
191.	−	+	+	−	+	v	−	−	−	−	−	+	n	−	+	−	+	v	−	+	−	+	+	+	+	−	−	n	−	−	+	+	−	−	−	9	51.8	−	−	−
192.	v	v	v	v	+	+	−	+	−	−	v	+	+	n	+	−	+	v	−	+	−	+	+	+	+	−	−	n	+	+	+	+	+	+	+	9	35.5	−	−	+
193.	+	−	+	−	+	+	−	−	−	−	−	+	−	−	+	−	−	−	n	+	−	+	+	+	n	−	−	n	+	+	+	+	−	−	−	9	47.5	−	−	−
194.	−	+	+	−	+	+	−	w	+	+	−	+	n	−	+	−	+	−	−	+	−	+	+	+	+	−	−	n	+	−	+	+	+	+	−	9	52.0	−	−	−
195.	+	+	+	v	+	+	+	+	+	+	−	v	n	−	−	−	+	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	55.3	−	−	−
196.	−	−	−	−	+	+	−	n	+	+	−	v	n	−	+	+	−	−	+	+	−	+	+	+	n	−	−	n	+	+	+	+	v	−	−	n	49.7	−	−	−
197.	+	+	+	+	+	v	−	−	+	+	+	−	−	n	+	−	+	+	+	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	n	48.8	−	n	−
198.	v	v	v	v	+	v	−	−	+	+	−	+	−	−	v	v	+	−	v	+	−	+	+	+	+	−	−	n	v	v	n	+	−	−	−	9	46.5	−	−	−
199.	−	−	−	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	9	44.1	−	−	−
200.	−	−	−	v	+	−	−	−	−	−	−	v	n	n	v	−	−	−	−	+	−	+	+	−	−	−	−	n	−	−	+	+	−	−	−	n	33.4	−	v	−
201.	+	+	+	−	+	+	−	+	−	−	−	+	n	n	+	v	+	−	−	+	−	+	+	+	+	−	−	n	+	+	+	+	+	+	v	n	45.9	−	−	v
202.	−	+	v	−	+	+	−	−	+	+	−	v	n	−	+	−	+	+	+	+	−	+	+	−	n	−	−	n	+	+	+	+	v	−	−	n	38.1	−	−	−
203.	+	v	−	−	+	+	−	n	−	−	−	+	n	−	+	+	v	−	−	+	−	+	+	−	−	−	−	n	+	+	+	+	−	−	−	n	48.3	−	−	−
204.	−	v	+	−	+	+	−	−	−	v	v	+	+	n	−	−	v	−	−	v	−	v	v	v	v	−	−	n	+	+	+	+	v	v	−	9	57.6	−	v	−
Cephaloascus																																								
205.	−	+	−	w	+	×	−	−	−	−	−	−	−	−	n	n	n	n	n	+	−	+	+	n	+	−	n	−	n	n	+	+	n	−	−	n	37.8	−	+	+
206.	−	−	−	v	×	×	−	−	−	−	+	−	−	−	n	n	n	n	n	+	−	+	+	n	v	−	n	−	n	n	+	+	n	−	−	n	39.9	−	+	+
Chionosphaera																																								
207.	−	−	−	−	−	−	−	−	−	n	−	−	n	n	n	n	n	n	n	n	n	n	n	n	n	+	v	n	n	n	+	+	−	−	−	10	51.6	+	−	v
Citeromyces																																								
208.	+	−	+	v	−	v	−	−	+	n	×	+	+	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	n	+	n	−	n	8	45.2	−	−	−
Clavispora																																								
209.	v	+	+	×	+	v	−	v	−	−	−	+	n	n	v	n	n	n	n	+	n	+	+	w	+	−	n	−	v	−	+	+	+	+	v	8	45.4	−	−	−
210.	−	+	+	+	+	+	−	+	−	−	−	+	n	n	v	n	n	n	n	+	n	v	+	−	v	−	n	−	−	−	+	+	+	+	+	8	43.6	−	−	−
Cryptococcus																																								
211.	+	+	+	−	+	+	+	−	+	n	+	+	v	+	n	n	n	n	n	n	n	n	n	−	−	−	+	n	n	n	+	+	n	−	n	10	54.9	+	−	−
212.	+	+	+	−	+	+	+	n	+	n	−	+	n	n	v	−	n	n	n	+	n	+	−	n	n	+	n	n	−	n	+	+	−	−	−	n	55.0	+	−	−
213.	v	+	+	v	+	+	+	−	+	n	−	+	v	+	n	n	n	n	n	n	n	n	n	−	−	×	+	−	n	n	+	+	n	v	n	10	53.7	+	−	−
214.	w	+	+	+	+	+	+	−	−	n	−	n	n	−	n	n	n	n	n	n	n	n	n	+	w	+	+	−	n	n	+	+	n	−	n	n	55.1	+	−	−
215.	+	+	+	−	+	v	+	n	+	n	+	+	n	n	−	−	n	n	n	+	−	+	v	n	n	+	n	n	−	n	+	−	−	−	−	n	55.0	+	−	−
216.	−	+	+	−	+	+	−	−	+	n	−	n	n	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	−	n	n	n	58.4	+	−	−
217.	+	×	+	−	+	×	+	−	−	n	−	+	+	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	10	48.6	+	−	−
218.	+	+	+	−	+	+	−	−	+	n	−	n	n	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	n	53.4	+	−	−
219.	−	+	+	−	−	−	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	−	n	n	n	n	56.0	+	−	−
220.	v	+	+	v	v	v	×	−	−	n	v	n	n	−	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	10	58.1	+	+	−
221.	−	+	+	w	+	w	+	−	−	n	−	+	+	w	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	9	53.1	+	+	−
222.	−	+	+	−	−	−	+	−	+	n	−	n	n	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	n	n	+	−	−
223.	+	+	+	w	+	w	+	−	−	n	−	+	+	w	n	n	n	n	n	n	n	n	n	−	+	−	+	w	n	n	+	+	n	−	n	10	55.0	+	−	−
224.	+	+	+	−	+	+	−	−	+	n	+	n	n	+	n	n	n	n	n	n	n	n	n	−	−	+	w	−	n	n	+	+	−	−	−	10	n	+	−	−
225.	−	+	+	−	v	+	+	−	+	n	−	n	n	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	n	52.4	+	−	−
226.	v	+	+	−	×	−	+	−	−	n	−	+	w	−	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	−	n	n	n	58.3	+	−	−
227.	−	+	+	−	+	−	+	n	−	−	−	+	−	n	−	−	+	n	n	+	−	+	−	n	n	+	n	n	+	n	n	+	n	n	n	9	n	+	n	n
228.	+	+	+	w	+	w	+	−	−	n	−	n	n	+	n	n	n	n	n	n	n	n	n	−	w	+	+	−	n	n	+	+	−	−	n	n	50.5	+	−	−
229.	−	+	+	w	+	w	−	−	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	+	+	+	−	n	n	+	w	−	n	n	n	n	+	−	−
230.	+	+	+	+	+	+	+	−	−	n	−	n	n	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	+	−	n	9	61.0	+	+	+
231.	v	+	+	w	+	+	+	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	−	n	n	n	10	56.5	+	−	−
232.	−	+	+	w	+	+	+	−	+	n	−	+	+	w	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	n	54.4	+	−	−
233.	+	×	+	v	+	×	+	−	−	n	v	+	+	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	+	−	n	10	57.0	+	+	−
234.	+	+	+	w	+	+	+	−	−	n	−	+	+	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	−	−	n	10	59.5	+	−	−
235.	−	×	+	v	+	+	+	−	+	n	−	+	+	−	n	n	n	n	n	n	n	n	n	−	−	+	+	+	n	n	+	+	−	n	n	8	61.2	+	−	−

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Cryptococcus (*cont'd*)																																			
236. *C. magnus*	–	–	–	–	–	–	–	+	w	v	+	+	+	+	+	–	+	+	–	w	w	+	v	+	v	–	–	–	–	v	–	–	w	+	+
237. *C. marinus*	–	–	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+
238. *C. podzolicus*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	+	–	–	–	+	+	+
239. *C. skinneri*	–	–	–	–	–	–	–	+	v	v	–	–	+	+	–	–	–	–	–	–	+	+	+	v	+	–	–	–	+	v	v	+	v	+	+
240. *C. terreus*	–	–	–	–	–	–	–	+	v	+	–	v	+	+	+	–	–	v	–	v	+	+	v	+	+	+	+	–	v	–	–	v	v	+	+
241. *C. vishniacii*	–	–	–	–	–	–	–	+	–	–	v	+	v	+	–	–	v	+	v	v	v	v	–	–	v	–	–	–	–	–	–	–	–	v	–
242. *C. yarrowii*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+	+	+	–	–	+	–	+	–	–	+	+	–	+	+	–	–	+	+	+
Cyniclomyces																																			
243. *C. guttulatus*	w	–	w	–	–	w	–	+	–	–	+	–	–	+	–	–	+	v	w	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cystofilobasidium																																			
244. *C. bisporidii*	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	–	×	+	–	–	–	+	v	–	v	v	+	+
245. *C. capitatum*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	×	–	+	+	v	v	+	v	+	v	v	–	–	–	–	+	–	–	+	+	+
246. *C. infirmominiatum*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	v	–	+	+	–	+	+	+	+	v	v	v	–	–	v	v	–	v	+	+	+
247. *C. lari-marini*	w	–	v	v	–	v	w	+	+	+	+	+	+	+	×	–	+	+	–	+	+	+	×	×	×	–	+	–	×	+	–	n	+	+	+
Debaryomyces																																			
248. *D. carsonii*	–	–	–	–	–	–	–	+	+	+	+	+	v	+	–	v	v	+	–	+	+	v	–	v	–	–	+	–	+	+	–	+	v	+	+
249. *D. castellii*	+	–	+	w	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	×	+	–	+	+	–	+	+	+	+
250. *D. coudertii*	–	–	–	–	–	–	–	+	+	–	–	+	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+
251. *D. etchellsii*	×	–	v	v	–	–	–	+	+	+	+	+	+	+	–	–	–	+	–	–	+	+	–	–	–	+	+	–	+	+	–	+	–	+	+
D. hansenii																																			
252. var. *hansenii*	v	v	v	v	–	v	v	+	+	v	+	+	+	+	v	v	+	v	v	v	+	×	v	v	v	v	v	–	×	+	v	+	v	+	×
253. var. *fabryi*	v	v	v	v	–	v	v	+	+	v	+	+	+	+	v	v	+	v	v	v	+	×	v	v	v	v	v	–	×	+	v	+	v	+	×
254. *D. maramus*	v	–	–	–	–	–	–	+	+	v	+	+	+	+	v	v	+	+	v	+	+	+	–	+	–	v	+	–	+	+	+	+	–	+	+
255. *D. melissophilus*	–	–	–	–	–	–	–	+	+	v	+	+	v	–	–	–	–	+	–	v	–	–	–	–	–	–	v	–	+	+	+	+	–	+	+
256. *D. nepalensis*	v	–	v	–	–	v	v	+	+	+	+	+	+	+	v	+	+	+	–	+	+	+	v	+	–	w	v	–	+	+	+	+	v	+	+
D. occidentalis																																			
257. var. *occidentalis*	+	+	+	v	–	v	–	+	+	v	+	+	+	+	v	v	+	+	+	+	+	v	–	–	–	w	+	–	+	+	–	v	–	+	v
258. var. *persoonii*	+	+	+	v	–	v	–	+	–	v	+	+	–	+	v	v	+	+	+	+	–	v	–	–	–	w	+	–	+	+	–	v	–	+	v
259. *D. polymorphus*	+	v	+	v	–	w	–	+	+	+	+	+	+	+	v	v	+	+	+	+	+	v	v	v	–	v	+	–	+	+	+	+	+	+	+
260. *D. pseudopolymorphus*	+	w	+	v	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	v	+	–	+	+	+	+	+	+	+
261. *D. robertsiae*	+	–	+	+	–	v	+	+	+	+	+	+	+	+	–	–	+	+	–	–	+	+	–	v	+	–	+	–	+	+	+	+	–	+	+
262. *D. udenii*	w	–	v	v	–	–	v	+	+	w	+	+	+	+	–	+	+	+	–	+	+	+	–	+	w	v	+	–	+	+	+	+	–	+	+
D. vanrijiae																																			
263. var. *vanrijiae*	v	–	v	–	–	–	–	+	+	+	+	+	v	+	–	+	+	+	v	+	+	v	–	v	v	v	+	–	+	+	+	+	+	+	+
264. var. *yarrowii*	v	–	v	–	–	–	–	+	+	+	+	+	v	v	–	–	+	+	v	v	+	v	–	v	v	v	+	–	+	+	v	+	+	+	+
265. *D. yamadae*	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	–	–	+	–	v	+	+	–	–	+	+	+	–	+	+	–	+	+	+	+
Dekkera																																			
266. *D. anomala*	+	+	+	v	v	v	v	+	+	–	+	v	+	+	v	–	v	v	–	–	–	–	–	–	–	×	n	–	v	+	–	–	–	–	–
267. *D. bruxellensis*	+	v	v	v	–	–	v	+	v	–	v	v	v	v	–	–	v	v	–	–	–	–	–	v	–	v	n	–	v	v	–	–	–	–	–
Dipodascopsis																																			
268. *D. tothii*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	–	–	n	–	+	+	–	+	–	+	+
D. uninucleata																																			
269. var. *uninucleata*	–	–	–	–	–	–	–	+	v	+	+	+	–	+	–	+	+	+	+	+	+	+	×	–	–	–	n	–	+	+	–	+	–	+	+
270. var. *wickerhamii*	–	–	–	–	–	–	–	+	–	+	–	+	–	+	–	+	–	+	–	+	+	+	+	–	+	–	n	–	+	+	–	+	–	+	×
Dipodascus																																			
271. *D. aggregatus*	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	v	–	–	n	n	+	+	–	+	–	+	+
272. *D. albidus*	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	n	n	+	+	–	–	–	+	+
273. *D. ambrosiae*	–	–	–	–	–	–	–	+	+	v	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	v	–	+	+
274. *D. armillariae*	–	–	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	n	n	v	+	–	–	–	+	v
275. *D. australiensis*	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	v	+	–	–	–	–	–	n	n	+	+	–	v	–	+	+
276. *D. capitatus*	–	–	–	–	–	–	–	+	+	v	–	–	–	–	–	–	–	–	–	–	–	–	–	v	–	–	n	n	+	+	–	–	–	–	–
277. *D. geniculatus*	v	–	–	–	–	–	n	+	+	+	–	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–	n	n	+	+	–	–	–	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Cryptococcus *(cont'd)*																																								
236.	+	v	+	−	w	w	+	−	−	n	−	n	n	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	10	51.6	+	−	−
237.	−	v	+	−	−	−	+	−	−	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	+	−	+	−	n	n	+	+	−	n	n	10	62.6	+	+	−
238.	+	+	+	−	w	+	+	−	−	n	+	n	n	+	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	−	n	n	n	62.8	+	−	+
239.	v	w	+	v	+	+	+	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	−	−	+	+	−	n	n	+	+	n	−	n	n	53.0	+	−	−
240.	v	+	+	v	w	v	+	−	+	n	+	+	+	+	n	n	n	n	n	n	n	n	n	−	w	+	+	−	n	n	+	+	−	n	n	10	57.5	+	−	−
241.	v	v	v	−	+	v	−	−	v	n	v	v	n	−	n	n	n	n	n	n	n	n	n	−	−	+	v	−	n	n	+	−	n	n	n	n	55.3	+	−	−
242.	+	−	+	−	w	w	+	−	+	n	−	+	n	w	−	−	−	+	−	+	−	×	+	−	w	−	+	−	−	−	+	+	−	n	n	10	60.3	+	−	−
Cyniclomyces																																								
243.	−	−	−	−	w	−	−	n	−	n	−	−	−	n	n	n	n	n	n	n	n	n	n	n	−	−	n	−	−	−	−	−	+	+	+	6	34.5	−	−	−
Cystofilobasidium																																								
244.	−	v	+	×	−	−	+	v	+	n	−	n	n	−	n	n	+	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	−	−	−	8	60.9	+	w	v
245.	−	+	+	×	−	−	+	−	+	n	−	n	n	−	n	n	−	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	−	−	−	8	56.6	+	−	+
246.	−	v	+	w	−	−	+	−	+	n	−	n	n	−	n	n	+	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	−	−	−	8	66.3	+	−	v
247.	−	×	n	−	+	−	+	−	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	+	n	×	+	n	n	−	n	n	n	+	−	n	n	+	+	+
Debaryomyces																																								
248.	+	+	−	v	+	+	−	×	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	v	n	n	+	+	n	v	n	9	39.7	−	+	−
249.	+	+	+	−	+	+	−	+	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	−	−	−	9	37.1	−	+	−
250.	−	+	+	−	+	+	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	−	−	−	9	37.4	−	−	−
251.	+	v	−	v	+	+	−	w	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	+	+	n	9	39.6	−	−	−
252.	+	×	×	v	+	v	−	v	−	v	−	+	v	−	+	v	n	−	n	+	n	+	+	n	+	−	n	n	n	n	+	+	v	−	−	9	38.4	−	v	−
253.	+	×	×	v	+	v	−	v	−	v	−	+	v	−	+	+	n	−	n	+	n	+	+	n	+	−	n	n	n	n	+	+	+	v	−	9	37.3	−	v	−
254.	+	+	+	−	+	+	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	v	−	−	9	39.1	−	v	−
255.	+	v	+	−	−	×	−	+	−	n	−	v	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	−	−	−	9	39.8	−	−	−
256.	+	+	+	v	+	+	−	−	−	+	−	+	−	−	+	+	n	v	n	+	n	+	+	n	+	−	n	n	n	n	+	+	+	−	−	9	37.8	−	v	−
257.	v	+	+	−	+	+	−	v	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	n	n	n	+	+	+	v	−	9	35.2	−	−	−
258.	v	−	+	−	+	+	−	v	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	n	n	n	+	+	+	v	−	9	35.4	−	−	−
259.	+	+	+	−	+	+	−	v	−	n	+	+	v	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	+	v	−	9	35.8	−	+	−
260.	+	+	+	+	+	+	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	−	−	−	9	35.7	−	+	−
261.	+	+	+	−	+	+	−	×	−	n	+	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	n	−	−	9	42.7	−	+	−
262.	+	+	+	−	+	+	−	+	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	−	−	−	9	35.8	−	+	−
263.	+	+	+	v	+	+	−	+	−	n	+	+	v	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	+	+	−	9	33.2	−	+	−
264.	+	v	+	v	+	v	−	+	−	n	+	+	v	−	n	n	n	n	n	n	n	n	n	n	+	−	n	n	n	n	+	+	+	−	−	9	31.8	−	+	−
265.	v	+	w	−	+	+	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	n	n	n	+	+	−	−	−	9	35.1	−	−	−
Dekkera																																								
266.	v	+	−	v	v	−	−	n	v	+	−	−	−	n	−	−	n	−	−	−	−	+	+	−	n	−	−	n	+	v	+	+	+	+	−	9	39.9	−	−	−
267.	v	v	−	v	−	−	−	n	v	v	−	v	−	n	−	−	n	−	−	−	−	+	+	−	n	−	−	n	+	v	+	+	+	+	v	9	39.4	−	v	−
Dipodascopsis																																								
268.	+	+	v	−	w	−	+	n	−	n	−	n	n	n	+	+	+	n	n	+	n	+	w	+	n	n	n	n	+	+	+	+	n	−	−	9	n	−	n	+
269.	+	−	v	−	−	−	+	n	−	n	−	n	n	n	+	+	−	n	n	+	n	+	w	+	n	n	n	n	+	+	+	+	+	+	n	9	n	−	n	+
270.	+	−	v	−	−	−	+	n	−	n	−	n	n	n	+	+	−	n	n	+	n	+	w	+	n	n	n	n	+	+	+	+	+	+	n	9	n	−	n	+
Dipodascus																																								
271.	−	−	−	v	+	+	−	n	−	n	−	−	n	n	n	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	n	45.6	−	n	+
272.	−	−	−	+	+	−	−	n	−	n	−	−	n	n	n	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	9	41.9	−	n	+
273.	−	−	−	+	+	−	−	n	−	n	−	−	n	n	n	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	n	45.2	−	n	+
274.	−	−	−	v	+	v	−	n	−	n	+	−	n	n	n	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	n	42.2	−	n	+
275.	−	−	−	+	+	−	−	n	−	n	−	−	n	n	n	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	+	n	40.0	−	n	+
276.	−	−	−	+	+	v	−	n	−	n	−	−	n	n	n	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	+	9	34.3	−	n	+
277.	−	−	−	+	+	+	−	n	−	n	−	−	n	n	n	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	n	43.2	−	n	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Dipodascus (*cont'd*)																																			
278. *D. ingens*	v	–	–	–	–	–	n	+	+	v	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	n	n	+	+	–	–	–	–	–
279. *D. macrosporus*	–	–	–	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	v	–	+	+
280. *D. magnusii*	+	×	+	–	–	×	n	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	n	n	–	+	–	–	–	+	+
281. *D. ovetensis*	v	–	–	–	–	–	n	+	+	v	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–	n	n	+	+	–	–	–	–	–
282. *D. spicifer*	–	–	–	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	n	n	+	+	–	–	–	–	–
283. *D. tetrasperma*	+	–	–	–	–	–	n	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	n	n	+	+	–	–	–	–	+
Eremothecium																																			
284. *E. ashbyi*	v	–	v	v	–	v	v	+	–	–	+	+	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	v
285. *E. coryli*	+	–	+	+	–	v	+	+	–	–	+	+	–	+	–	–	+	–	–	v	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
286. *E. cymbalariae*	–	–	–	–	–	–	–	+	v	–	+	×	–	+	–	v	+	v	–	v	–	–	–	–	–	–	–	–	v	+	–	–	–	–	–
287. *E. gossypii*	–	–	–	–	–	–	–	+	–	–	+	×	v	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	v	+	–	–	–	–	v
288. *E. sinecaudum*	v	–	v	v	–	–	v	+	–	–	+	+	–	v	–	–	–	–	–	+	–	–	–	v	–	–	–	–	–	+	–	–	–	–	+
Erythrobasidium																																			
289. *E. hasegawianum*	–	–	–	–	–	–	–	+	+	+	+	w	+	+	–	–	–	+	–	–	+	+	+	+	–	+	n	–	–	+	–	+	–	+	+
Fellomyces																																			
290. *F. fuzhouensis*	–	–	–	–	–	–	–	+	+	+	+	v	+	+	+	+	v	v	–	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
291. *F. penicillatus*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	w	w	+	–	+	+	+	+	+	+	+	+	–	–	w	+	+	+	+	+
292. *F. polyborus*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	v	+	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+
Fibulobasidium																																			
293. *F. inconspicuum*	–	–	–	–	–	–	–	+	v	+	+	+	+	+	+	–	+	+	–	v	+	+	+	+	+	+	n	–	+	+	+	+	+	+	+
Filobasidiella																																			
F. neoformans																																			
294. var. *neoformans*	–	–	–	–	–	–	–	+	+	–	+	+	×	+	–	–	×	+	v	+	+	×	+	v	+	v	v	–	w	–	–	v	+	+	+
295. var. *bacillispora*	–	–	–	–	–	–	–	+	+	–	+	+	×	+	–	–	×	+	v	+	+	×	+	v	+	v	v	–	w	–	–	v	+	+	+
Filobasidium																																			
296. *F. capsuligenum*	+	–	–	v	–	–	–	+	+	–	+	+	×	+	–	–	–	–	+	+	v	v	v	v	–	v	v	–	w	×	–	+	v	+	+
297. *F. elegans*	–	–	–	–	–	–	–	+	–	–	+	+	–	+	–	–	–	–	–	w	+	+	–	–	–	–	–	–	–	–	–	–	–	+	v
298. *F. floriforme*	–	–	–	–	–	–	–	+	×	–	+	+	+	+	+	–	+	+	v	+	×	×	×	v	+	+	+	–	+	v	–	+	v	+	+
299. *F. globisporum*	–	–	–	–	–	–	–	+	–	–	–	+	+	+	+	–	–	–	–	w	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–
300. *F. uniguttulatum*	–	–	–	–	–	–	–	+	v	–	+	+	–	+	–	–	+	+	+	v	+	+	v	–	v	–	+	–	–	w	–	v	–	+	+
Galactomyces																																			
301. *G. citri-aurantii*	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	+	v	–	–	–	–	n	n	+	+	–	+	–	+	+
302. *G. geotrichum*	v	v	–	–	–	–	n	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	v	–	–	n	n	+	+	–	v	–	v	+
303. *G. reessii*	v	–	–	–	–	–	n	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	n	n	+	+	–	–	–	–	+
Geotrichum																																			
304. *G. clavatum*	–	–	–	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	n	–	–	–	n	+	+	–	–	–	–	–
305. *G. fermentans*	+	+	–	–	–	–	n	+	+	+	–	–	+	–	–	–	–	–	–	–	+	v	v	+	–	–	n	n	+	+	–	v	–	+	+
306. *G. fragrans*	+	v	–	–	–	–	n	+	+	+	–	–	–	–	–	–	–	–	–	–	–	n	–	–	–	–	–	n	+	+	–	–	–	v	v
307. *G. klebahnii*	+	v	–	–	–	–	n	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	n	+	+	–	v	–	+	+
Hanseniaspora																																			
308. *H. guilliermondii*	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	n	–	–	–	–	–	–	–	–
309. *H. occidentalis*	+	–	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	n	–	–	+	–	–	–	–	–
310. *H. osmophila*	+	–	–	–	–	–	–	+	–	–	v	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	n	–	–	–	–	–	–	–	–
311. *H. uvarum*	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	n	–	–	–	–	–	–	–	v
312. *H. valbyensis*	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	n	–	–	–	–	–	–	–	–
313. *H. vineae*	+	–	–	–	–	–	–	+	–	–	v	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	n	–	–	–	–	–	–	–	–
Holtermannia																																			
314. *H. corniformis*	–	–	–	–	–	–	–	+	+	+	v	+	+	+	–	–	–	v	–	+	+	+	+	v	+	–	n	–	+	v	–	+	+	+	+
Hyalodendron																																			
315. *H. lignicola*	–	–	–	–	–	–	–	+	+	–	+	+	+	+	+	+	+	+	–	v	+	+	v	+	+	n	n	n	n	+	+	+	–	+	+
Issatchenkia																																			
316. *I. occidentalis*	+	–	–	–	–	–	–	+	–	v	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	×	–	–	–	–	–

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
***Dipodascus* (cont'd)**																																								
278.	–	–	–	+	+	–	–	n	–	n	+	–	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	v	n	n	39.5	–	n	–
279.	–	–	–	v	+	+	–	n	–	n	+	–	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	–	–	n	41.5	–	n	+
280.	–	–	–	v	+	–	–	n	–	n	–	–	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	–	–	9	37.5	–	n	+
281.	–	–	v	+	+	–	–	n	–	n	v	–	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	–	–	9	51.3	–	n	+
282.	–	+	–	+	+	+	–	n	–	n	–	–	n	n	n	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	n	n	30.6	–	n	+
283.	–	–	–	+	+	+	–	–	–	n	–	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	n	n	38.4	–	n	+
Eremothecium																																								
284.	–	–	–	–	v	v	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	w	+	+	+	+	+	+	n	6	n	–	v	+
285.	v	–	–	–	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	v	+	+	+	+	+	+	+	5/6	43.3	–	–	+
286.	+	–	–	–	v	–	–	–	–	–	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	+	+	+	+	+	+	n	7	n	–	–	+
287.	–	v	v	–	×	w	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	v	+	+	+	+	+	×	n	6	n	–	v	+
288.	–	–	–	–	–	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	+	+	+	+	+	+	+	9	41.8	–	–	+
Erythrobasidium																																								
289.	–	+	–	+	+	+	–	n	+	n	–	+	n	n	n	n	+	n	n	n	n	n	n	n	n	–	+	n	n	n	+	+	n	–	–	10(H_2)	53.5	+	–	+
Fellomyces																																								
290.	–	+	+	–	+	+	+	–	–	n	–	+	+	n	n	n	+	n	n	n	n	n	–	–	w	v	×	n	n	n	+	+	n	–	–	10	54.6	+	+	v
291.	w	w	+	v	+	+	+	–	–	n	–	+	+	n	n	n	w	n	n	n	n	n	–	–	n	w	+	n	+	+	+	+	n	–	–	10	48.9	+	n	v
292.	+	×	+	w	+	+	+	–	–	n	–	+	+	n	n	n	+	n	n	n	n	n	–	–	+	–	+	n	+	+	+	+	n	–	–	10	49.6	+	–	–
Fibulobasidium																																								
293.	+	w	+	+	+	v	+	n	v	+	–	+	n	n	n	n	n	n	n	n	n	n	n	–	n	+	+	n	n	n	+	+	n	–	–	n	n	+	n	v
Filobasidiella																																								
294.	+	+	+	–	v	v	+	–	–	–	–	n	n	v	n	n	+	n	n	n	n	n	n	–	n	+	+	n	+	+	+	+	+	+	v	10	55.0	+	–	+
295.	+	+	+	–	v	v	+	–	–	–	–	n	n	v	n	n	+	n	n	n	n	n	n	–	n	+	+	n	+	+	+	+	+	v	–	10	56.8	+	–	+
Filobasidium																																								
296.	+	+	v	–	+	+	+	v	–	–	–	–	n	–	n	n	n	n	n	n	–	+	+	–	n	+	+	n	v	n	+	+	n	v	–	10	49.8	+	–	v
297.	–	–	–	–	v	v	–	n	–	–	–	–	n	–	n	n	n	n	n	n	–	–	–	n	n	+	+	n	–	n	+	+	n	–	–	n	n	+	n	v
298.	+	+	+	–	+	+	+	–	+	+	–	–	n	+	n	n	n	n	n	n	–	v	+	–	n	+	+	n	–	n	+	+	n	–	–	10	n	+	–	v
299.	–	+	+	–	–	v	+	n	–	–	–	+	n	–	n	n	n	n	n	n	–	–	–	n	n	+	+	n	–	n	+	+	n	–	–	n	n	+	n	v
300.	+	+	+	v	v	v	+	w	–	–	–	+	n	–	n	n	n	n	n	n	–	×	–	–	n	+	+	n	–	n	+	+	n	–	–	9	n	+	–	v
Galactomyces																																								
301.	–	–	–	+	+	+	–	n	–	n	–	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	–	–	n	38.6	–	n	+
302.	–	–	–	v	v	v	–	n	–	n	+	–	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	v	n	n	43.6	–	n	+
303.	–	–	–	+	+	v	–	n	–	n	–	–	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	v	n	n	35.7	–	n	+
Geotrichum																																								
304.	–	+	–	v	+	+	–	n	–	n	–	–	n	n	n	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	n	n	32.1	–	n	+
305.	–	×	–	+	+	+	–	n	–	n	+	–	n	n	n	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	v	n	n	44.7	–	n	+
306.	–	–	–	+	+	v	–	n	–	n	–	–	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	v	n	n	43.2	–	n	+
307.	–	–	–	+	+	v	–	n	–	n	+	–	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	–	–	n	40.4	–	n	+
Hanseniaspora																																								
308.	–	+	v	–	–	–	–	n	–	–	–	+	+	n	–	–	–	–	–	+	–	+	+	v	n	–	–	n	+	+	+	+	+	+	–	6	33.5	–	–	–
309.	–	+	–	–	–	–	–	n	–	–	–	–	–	n	–	–	v	–	–	+	–	+	+	–	n	–	–	n	–	–	+	+	n	–	–	6	35.4	–	–	–
310.	–	+	–	–	–	–	–	n	–	–	–	–	–	n	–	–	+	–	–	+	–	+	+	v	n	–	–	n	–	–	+	+	–	–	–	6	40.1	–	–	–
311.	–	+	v	–	–	–	–	n	–	–	–	+	–	n	–	–	+	–	–	+	–	+	+	v	n	–	–	n	+	+	+	+	n	–	–	6	33.9	–	–	–
312.	–	+	–	–	–	–	–	n	–	–	–	–	–	n	–	–	+	–	–	+	–	+	+	–	n	–	–	n	+	+	+	+	–	–	–	6	29.4	–	–	–
313.	–	+	–	–	–	–	–	n	–	–	–	–	–	n	–	–	+	–	–	+	–	+	+	+	n	–	–	n	–	–	+	+	+	–	–	6	38.7	–	–	–
Holtermannia																																								
314.	v	+	+	+	+	+	+	n	–	–	v	+	n	n	n	n	n	n	n	n	n	n	n	–	n	+	+	n	n	n	+	+	v	v	n	n	n	+	n	v
Hyalodendron																																								
315.	×	+	n	v	+	+	v	n	–	n	w	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	+	n	–	–	9	n	×	n	+
Issatchenkia																																								
316.	–	–	–	×	+	–	–	–	–	n	+	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	n	+	+	+	–	7	41.0	–	+	–

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Issatchenkia (*cont'd*)																																			
317. *I. orientalis*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	−	−	−	−
I. scutulata																																			
318. var. *scutulata*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−
319. var. *exigua*	×	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−
320. *I. terricola*	×	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−
Itersonilia																																			
321. *I. perplexans*	−	−	−	−	−	−	−	+	+	−	+	v	+	+	v	v	+	+	v	+	+	v	v	v	v	+	n	−	v	v	−	v	−	+	v
Kloeckera																																			
322. *K. lindneri*	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	n	−	−	−	−	−	−	−	−
Kluyveromyces																																			
323. *K. aestuarii*	+	v	+	−	−	+	−	+	+	+	+	v	+	v	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+	+
324. *K. africanus*	+	+	−	−	−	−	−	+	×	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−
325. *K. bacillisporus*	+	−	−	−	−	−	−	+	−	−	×	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	w	−	−	−	−	−
326. *K. blattae*	+	+	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−
327. *K. delphensis*	+	−	−	−	−	−	−	+	−	−	−	−	−	w	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
328. *K. dobzhanskii*	+	+	+	+	−	+	+	+	+	v	+	+	+	+	−	−	v	+	−	−	v	v	−	−	−	−	−	−	+	v	−	v	−	+	+
K. lactis																																			
329. var. *lactis*	+	+	v	v	+	v	v	+	+	v	+	v	+	+	+	−	v	+	v	−	v	−	−	−	−	−	−	−	+	v	−	v	−	+	+
330. var. *drosophilarum*	+	+	v	v	−	v	v	+	+	v	+	v	v	v	−	−	v	v	v	−	v	v	−	−	−	−	−	−	+	v	−	v	−	v	+
331. *K. lodderae*	+	+	+	−	−	+	+	+	+	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−
332. *K. marxianus*	+	+	+	−	v	+	−	+	+	v	+	−	v	v	v	−	+	−	+	−	+	v	−	v	−	−	−	−	+	+	−	+	−	v	v
333. *K. phaffii*	+	+	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	+	−	−	−	−	−
334. *K. polysporus*	+	+	+	−	−	+	+	+	+	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	v	+	−	−	−	−	−
335. *K. thermotolerans*	+	v	+	v	−	+	+	+	+	v	+	+	−	+	−	v	+	+	v	−	−	−	−	−	−	−	−	−	+	v	−	v	−	+	v
336. *K. waltii*	+	−	+	−	−	+	−	+	−	+	+	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	−	+	+
337. *K. wickerhamii*	+	+	+	−	−	−	−	+	+	v	+	−	×	v	+	−	v	−	−	−	+	−	−	−	−	−	−	−	×	v	−	−	−	−	w
338. *K. yarrowii*	+	+	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	−	−	w	v	−	−	−	−	−
Kockovaella																																			
339. *K. imperatae*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	v	+	+	×
340. *K. thailandica*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	v	−	v	−	+	+
Kurtzmanomyces																																			
341. *K. nectairei*	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	w	−	+	+
342. *K. tardus*	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	n	−	+	−	−	−	−	+	+
Leucosporidium																																			
343. *L. antarcticum*	−	−	−	−	−	−	−	+	v	−	v	v	−	v	−	−	v	−	−	−	v	−	−	−	−	−	−	−	v	×	−	−	−	v	−
344. *L. fellii*	−	−	−	−	−	−	−	+	−	+	w	−	+	+	+	−	−	−	−	−	+	−	+	−	+	+	+	−	+	+	−	+	−	+	+
345. *L. scottii*	−	−	−	−	−	−	−	+	v	+	+	+	w	+	v	−	+	+	−	−	+	v	v	v	+	+	+	−	+	+	−	v	v	+	+
Lipomyces																																			
346. *L. japonicus*	−	−	−	−	−	−	−	+	+	+	+	−	+	−	+	−	−	−	−	−	+	+	−	−	−	−	n	−	v	+	−	+	−	+	+
L. kononenkoae																																			
347. ssp. *kononenkoae*	−	−	−	−	−	−	−	+	+	+	+	+	v	+	−	+	+	+	+	+	+	−	v	−	−	−	n	−	+	v	−	−	+	+	+
348. ssp. *spencer-martinsiae*	−	−	−	−	−	−	−	+	+	+	+	+	v	v	−	+	+	+	+	+	−	−	v	−	−	−	n	−	v	+	−	−	−	+	+
349. *L. lipofer*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	v	+	+	+	+	+	v	v	v	−	−	−	n	−	×	v	+	v	v	+	+
350. *L. starkeyi*	−	−	−	−	−	−	−	+	v	v	+	v	v	v	v	+	v	v	v	v	v	v	v	v	v	−	n	−	v	v	v	v	v	v	v
351. *L. tetrasporus*	−	−	−	−	−	−	−	+	+	+	+	+	v	v	v	+	+	+	+	+	+	+	+	v	v	−	n	−	+	v	+	+	v	+	+
Lodderomyces																																			
352. *L. elongisporus*	+	−	−	−	−	−	+	+	+	+	+	+	−	+	−	−	−	+	−	−	×	−	−	−	−	−	+	−	+	+	−	+	−	+	+
Metschnikowia																																			
353. *M. agaves*	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	−	−	w	v	−	−	−	−	+	−	+	−	−	+	−	+	+
354. *M. australis*	−	−	−	−	−	−	−	+	×	v	+	+	+	+	−	−	−	w	−	−	+	−	−	w	−	−	+	−	+	+	−	−	−	+	+
M. bicuspidata																																			
355. var. *bicuspidata*	+	−	−	−	−	−	−	+	+	v	+	+	+	+	−	−	−	v	−	−	v	−	−	−	−	−	+	−	×	v	−	−	−	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine
***Issatchenkia* (*cont'd*)**																					
317.	−	−	−	+	+	×	−	−	−	n	+	−	−	−	n	n	n	n	n	n	n
318.	−	−	−	×	+	−	−	−	−	n	+	−	−	−	n	n	n	n	n	n	n
319.	−	−	−	×	+	−	−	−	−	n	+	−	−	−	n	n	n	n	n	n	n
320.	−	−	−	−	+	v	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n
Itersonilia																					
321.	−	v	v	−	+	v	v	n	v	v	−	+	n	n	v	−	+	−	−	−	−
Kloeckera																					
322.	−	+	v	−	−	−	−	n	−	−	−	−	−	n	−	−	+	−	−	+	−
Kluyveromyces																					
323.	−	+	−	+	+	−	−	−	−	−	−	−	n	n	−	n	n	n	n	+	n
324.	−	−	−	−	−	−	−	−	−	−	−	−	n	n	−	n	n	n	n	−	n
325.	−	−	+	−	−	−	−	−	−	−	−	+	n	n	−	n	n	n	n	−	n
326.	−	−	−	−	−	−	−	−	−	−	−	+	n	n	−	n	n	n	n	−	n
327.	−	−	+	w	−	−	−	−	−	−	−	−	n	n	−	n	n	n	n	−	n
328.	+	+	−	+	+	v	−	−	−	−	−	−	n	n	v	n	n	n	n	+	n
329.	v	+	−	v	+	−	−	−	−	−	−	−	n	n	v	n	n	n	n	v	n
330.	v	v	−	v	+	v	−	−	−	−	−	−	n	n	v	n	n	n	n	+	n
331.	−	−	−	+	v	−	−	−	−	−	−	−	n	n	−	n	n	n	n	+	n
332.	−	v	−	+	+	v	−	−	−	−	−	−	n	n	v	n	n	n	n	+	n
333.	−	−	+	−	−	−	−	−	−	−	−	−	n	n	−	n	n	n	n	−	n
334.	−	−	+	v	w	+	−	−	−	−	−	−	n	n	−	n	n	n	n	−	n
335.	+	−	−	−	v	−	−	−	−	−	−	v	n	n	+	n	n	n	n	+	n
336.	−	−	−	−	−	−	−	−	−	−	−	−	n	n	+	n	n	n	n	+	n
337.	−	+	−	v	v	−	−	−	−	−	−	−	n	n	+	n	n	n	n	+	n
338.	−	−	−	−	−	−	−	−	−	−	−	−	n	n	×	n	n	n	n	−	n
Kockovaella																					
339.	−	×	+	−	×	v	w	−	−	−	−	×	w	n	n	n	n	n	n	−	n
340.	v	v	−	−	w	v	v	−	−	−	−	+	+	n	n	n	n	n	n	−	n
Kurtzmanomyces																					
341.	−	w	−	−	+	−	−	−	+	n	−	+	+	n	n	n	−	n	n	n	n
342.	−	−	−	−	−	−	−	n	+	+	−	n	n	n	n	n	−	n	n	−	−
Leucosporidium																					
343.	−	−	+	−	−	−	−	−	+	n	+	+	−	−	n	n	n	n	n	n	n
344.	−	+	+	w	w	w	−	−	+	n	+	n	n	−	n	n	+	n	n	+	−
345.	+	+	+	−	−	−	−	−	+	n	+	+	+	v	n	n	n	n	n	n	n
Lipomyces																					
346.	−	v	−	−	−	+	−	n	−	n	−	n	n	n	n	n	n	n	+	n	n
347.	+	v	v	−	+	v	−	n	−	n	+	n	n	n	n	n	n	n	n	n	n
348.	+	v	v	−	−	v	−	n	−	n	+	n	n	n	n	n	n	n	n	n	n
349.	+	+	+	−	×	×	−	n	−	n	+	n	n	n	n	n	n	n	+	n	n
350.	v	v	v	−	v	v	v	n	−	n	+	n	n	n	n	n	n	n	v	n	n
351.	+	v	v	−	v	w	v	n	−	n	+	n	n	n	n	n	n	n	+	n	n
Lodderomyces																					
352.	+	−	×	−	+	+	−	+	−	n	−	+	+	−	n	n	n	n	n	n	n
Metschnikowia																					
353.	−	+	w	−	+	+	−	w	−	n	−	v	−	n	n	n	n	n	n	+	n
354.	−	w	−	−	+	−	−	−	−	−	−	−	−	n	n	n	n	n	n	w	n
355.	−	×	+	−	v	−	−	−	−	−	−	+	−	n	n	n	n	n	n	+	n

	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
***Issatchenkia* (*cont'd*)**																			
317.	n	n	n	+	−	n	v	n	n	n	+	+	+	+	7	40.0	−	+	−
318.	n	n	n	+	−	n	−	n	n	n	+	n	−	−	7	32.8	−	+	−
319.	n	n	n	−	−	n	−	n	n	n	+	n	−	−	7	32.5	−	+	−
320.	n	n	n	v	−	n	−	n	n	n	+	n	v	−	7	40.1	−	+	−
Itersonilia																			
321.	v	−	−	n	−	+	n	−	−	+	+	−	−	−	9	62.0	+	−	+
Kloeckera																			
322.	+	+	−	n	−	−	n	+	+	+	+	−	−	−	n	n	−	n	−
Kluyveromyces																			
323.	+	+	−	+	−	n	−	−	−	+	+	n	−	−	n	39.8	−	+	−
324.	−	−	−	−	−	n	−	−	−	+	+	n	v	n	n	38.5	−	−	−
325.	−	−	+	+	−	n	−	−	−	+	+	+	w	−	−	38.0	−	−	−
326.	−	−	−	−	−	n	−	−	−	+	+	n	−	−	n	34.2	−	−	−
327.	w	−	w	−	−	n	−	−	−	+	+	×	+	n	n	40.2	−	−	−
328.	+	+	v	−	−	n	−	+	n	+	+	n	v	n	n	42.6	−	−	−
329.	v	v	v	−	−	n	−	+	n	+	+	n	v	n	6	40.4	−	v	−
330.	+	+	v	−	−	n	−	+	n	+	+	+	+	n	6	40.4	−	v	−
331.	−	v	−	−	−	n	−	+	n	+	+	n	−	−	n	35.6	−	−	−
332.	+	+	−	−	−	n	−	+	n	+	+	+	+	n	6	41.3	−	v	−
333.	−	−	−	−	−	n	−	−	−	+	+	n	−	−	n	35.3	−	−	−
334.	−	−	−	−	−	n	−	−	−	+	+	+	v	n	n	35.3	−	+	−
335.	+	+	+	−	−	n	−	−	−	+	+	+	v	n	6	46.2	−	−	−
336.	+	+	w	−	−	n	−	+	n	+	+	+	v	n	n	45.7	−	−	−
337.	+	+	−	−	−	n	−	+	n	+	+	+	v	n	n	42.2	−	+	−
338.	−	−	−	−	−	n	−	−	−	+	+	n	−	−	6	34.5	−	−	−
Kockovaella																			
339.	+	−	−	n	−	+	w	n	n	+	+	−	−	−	10	50.7	+	v	+
340.	+	−	−	n	−	+	w	n	n	+	+	−	−	−	10	48.5	+	+	+
Kurtzmanomyces																			
341.	n	+	−	n	−	+	n	−	−	+	+	−	−	−	10	52.5	+	−	−
342.	n	+	−	−	−	+	n	−	−	+	+	n	−	−	10	58.2	+	−	+
Leucosporidium																			
343.	n	n	−	−	−	+	+	n	n	−	n	n	n	n	10	50.5	+	−	v
344.	+	+	−	w	−	+	−	n	n	+	+	−	n	n	10	57.7	+	−	+
345.	n	+	−	+	−	+	+	n	n	+	+	n	−	n	9/10	60.0	+	−	v
Lipomyces																			
346.	n	n	n	n	+	n	n	+	+	+	+	n	−	−	9	41.9	−	−	−
347.	n	n	n	n	−	n	n	+	+	+	+	+	v	−	9	48.3	−	v	−
348.	n	n	n	n	−	n	n	+	+	+	+	+	v	+	9	n	−	v	−
349.	n	n	n	n	×	n	n	n	−	+	+	n	−	−	10	48.2	−	−	−
350.	n	n	n	n	v	n	n	+	+	+	+	n	−	−	9	47.5	−	−	−
351.	n	n	n	n	×	n	n	+	+	+	+	n	n	−	9	48.5	−	v	−
Lodderomyces																			
352.	n	n	n	+	−	n	−	n	n	+	+	+	+	n	9	39.7	−	−	−
Metschnikowia																			
353.	+	+	+	+	−	−	−	−	−	+	+	+	+	n	n	n	−	−	−
354.	+	+	−	+	−	−	−	−	−	+	+	−	−	−	9	47.0	−	−	−
355.	+	+	−	+	−	−	−	−	−	+	+	−	−	−	9	48.0	−	−	−

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
M. bicuspidata (*cont'd*)																																			
356. var. *californica*	+	−	−	−	−	−	−	+	+	v	+	+	+	+	−	−	−	v	−	−	v	−	−	−	−	−	+	−	×	v	−	−	−	+	+
357. var. *chathamia*	+	−	−	−	−	−	−	+	+	v	+	+	+	+	−	−	−	v	−	−	v	−	−	−	−	−	+	−	×	v	−	×	−	+	+
358. *M. gruessii*	+	−	−	−	−	−	−	+	v	+	+	+	+	−	−	−	−	+	−	−	v	−	−	v	−	+	+	−	w	+	−	−	−	w	−
359. *M. hawaiiensis*	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	−	−	+	−	−	−	−	v	+	−	+	+	−	×	−	+	+
360. *M. krissii*	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	×	+	−	−	−	+	−
361. *M. lunata*	+	−	−	−	−	−	−	+	+	−	+	+	+	+	−	−	−	+	−	−	+	−	−	−	−	w	+	−	w	+	−	w	−	+	+
362. *M. pulcherrima*	+	v	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+	−	−	+	−	−	v	−	−	+	−	+	+	−	v	−	+	+
363. *M. reukaufii*	+	v	−	−	−	−	−	+	v	v	+	+	+	+	−	−	−	+	−	−	v	−	−	v	−	v	+	−	w	+	−	+	−	+	+
364. *M. zobellii*	×	w	−	−	−	−	−	+	+	v	+	+	+	+	−	−	−	+	−	−	v	−	−	−	−	×	−	−	+	+	−	+	−	+	+
Moniliella																																			
365. *M. acetoabutens*	+	−	+	+	−	−	n	+	−	−	+	+	+	−	+	−	−	−	−	−	−	−	−	v	−	n	n	n	n	+	+	v	−	+	+
366. *M. mellis*	+	+	−	+	−	−	n	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	+	−	n	n	n	n	n	w	−	−	+	+
367. *M. pollinis*	+	w	+	+	−	−	n	+	−	−	+	+	+	−	−	−	−	−	−	v	v	−	−	+	−	n	n	n	n	+	+	−	−	+	n
368. *M. suaveolens*	+	v	+	+	v	−	n	+	v	−	+	+	+	−	v	−	v	v	−	v	v	v	−	+	−	n	n	n	n	+	+	v	−	+	v
Mrakia																																			
369. *M. frigida*	+	v	v	v	−	v	−	+	v	+	+	v	+	+	v	v	+	v	−	v	+	+	v	v	v	+	+	−	+	v	−	+	v	+	+
Myxozyma																																			
370. *M. geophila*	−	−	−	−	−	−	−	+	+	+	×	−	−	−	v	−	−	−	−	−	+	+	+	+	−	−	−	−	+	+	−	+	+	+	+
371. *M. kluyveri*	−	−	−	−	−	−	−	+	+	+	+	−	+	+	+	+	+	−	v	−	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+
372. *M. lipomycoides*	−	−	−	−	−	−	−	+	+	+	−	−	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−	+	+	−	+	−	−	+
373. *M. melibiosi*	−	−	−	−	−	−	−	+	+	v	−	−	+	−	v	+	−	−	−	−	+	+	+	+	−	−	−	−	+	+	−	+	−	v	+
374. *M. monticola*	−	−	−	−	−	−	−	+	+	+	+	+	−	+	+	+	v	+	−	−	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+
375. *M. mucilagina*	−	−	−	−	−	−	−	+	+	+	+	+	+	v	−	−	−	+	−	−	+	+	v	v	+	−	−	−	+	+	−	+	−	+	+
376. *M. udenii*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+	−	−	−	−	−	−	+	+	−	+	−	+	+
377. *M. vanderwaltii*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	v	−	−	+	−	−	+	+	+	v	−	−	−	−	+	+	−	+	−	−	+
Nadsonia																																			
378. *N. commutata*	−	−	−	−	−	−	−	+	−	−	−	v	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	v
N. fulvescens																																			
379. var. *fulvescens*	+	+	+	×	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	v	−	+	+
380. var. *elongata*	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	v	−	−	+
Oosporidium																																			
381. *O. margaritiferum*	−	−	−	−	−	−	−	+	v	+	+	+	−	+	−	−	+	+	−	+	−	v	−	−	−	−	n	n	+	−	−	−	−	v	v
Pachysolen																																			
382. *P. tannophilus*	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−	−	−	−	+	+	−	v	−	−	−	−	+	+	−	+	−	+	+
Pichia																																			
383. *P. acaciae*	+	v	−	+	−	−	+	+	+	v	−	+	+	+	v	−	−	−	−	−	×	+	−	+	−	−	+	−	+	+	+	+	−	+	+
384. *P. alni*	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+	−	−	v	+	−	−	−	+	+	−	v	−	+	+
385. *P. americana*	×	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+	−	−	−	+	−	−	−	+	+	−	−	−	+	+
P. amethionina																																			
386. var. *amethionina*	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	−	−	+	+	−	−	−	−	−
387. var. *pachycereana*	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+	−
388. *P. amylophila*	+	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	+	+	+	−	−	−	−	−	−	+	+	−	−	−	+	+
389. *P. angophorae*	+	−	+	+	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+	+	−	−	−	+	+	−	+	−	−	+	−	+	+
390. *P. angusta*	+	−	−	−	−	−	+	+	v	v	+	+	v	+	−	−	−	+	−	−	v	v	v	+	v	−	−	+	+	+	+	+	v	+	+
391. *P. anomala*	+	v	+	v	−	v	−	+	v	−	+	+	+	+	−	−	+	+	−	+	v	v	−	v	−	−	−	−	+	+	+	v	−	+	+
392. *P. antillensis*	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	−	−	−	+	−
393. *P. barkeri*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	+	+	−	+	+	−	−	−	−	−
394. *P. besseyi*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	+
395. *P. bimundalis*	+	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+	v	+	−	+	−	−	−	+	+	−	−	−	+	+
396. *P. bispora*	v	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+	v	−	v	+	−	−	−	+	+	−	v	−	v	×
397. *P. bovis*	+	−	v	v	−	−	v	+	−	−	+	+	+	+	−	−	−	+	−	v	+	+	−	−	v	−	−	−	+	+	−	−	−	+	+
398. *P. burtonii*	+	v	+	×	−	×	+	+	+	v	+	+	+	+	−	−	+	v	−	+	+	v	−	+	−	×	+	−	+	+	+	+	−	+	+

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
M. bicuspidata (cont'd)																																								
356.	+	×	+	−	v	−	−	−	−	−	−	+	−	n	n	n	n	n	n	+	n	+	+	−	+	−	−	−	−	−	+	+	n	−	−	9	47.6	−	−	−
357.	+	×	−	−	v	−	−	−	−	−	−	−	−	n	n	n	n	n	n	+	n	+	+	−	+	−	−	−	−	−	+	+	−	−	−	9	47.5	−	−	−
358.	−	+	v	−	w	−	−	−	−	n	−	−	−	n	n	n	n	n	n	+	n	+	+	−	+	−	−	−	−	−	+	+	−	−	−	9	39.2	−	−	−
359.	−	+	w	−	+	+	−	×	−	−	−	+	−	n	n	n	n	n	n	+	n	+	+	+	+	−	−	−	−	−	+	+	n	−	−	n	46.7	−	−	+
360.	+	+	−	−	+	−	−	−	−	−	−	−	−	n	n	n	n	n	n	+	n	+	+	−	+	−	−	−	−	−	+	+	n	−	−	9	45.4	−	−	−
361.	v	+	+	−	+	−	−	+	−	−	−	+	−	n	n	n	n	n	n	+	n	+	+	v	+	−	−	−	−	−	+	+	n	−	−	9	44.2	−	−	−
362.	+	+	+	v	+	v	−	+	−	−	−	+	−	n	n	n	n	n	n	+	n	+	+	+	+	−	−	−	−	−	+	+	n	v	n	9	45.6	−	−	−
363.	v	+	v	−	+	−	−	w	−	−	−	+	−	n	n	n	n	n	n	+	n	+	+	+	+	−	−	−	−	−	+	+	n	−	−	9	41.3	−	−	−
364.	+	+	−	−	+	−	−	−	−	−	−	−	−	n	n	n	n	n	n	+	n	+	+	w	+	−	−	−	+	−	+	+	n	−	−	9	47.5	−	v	−
Moniliella																																								
365.	−	−	n	−	v	v	−	n	+	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	+	+	n	n	61.2	+	n	+
366.	−	−	n	−	w	w	−	n	+	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	+	n	−	−	n	58.7	+	n	+
367.	−	−	n	−	+	n	−	n	+	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	+	+	+	n	n	50.4	+	n	+
368.	−	v	n	−	+	v	−	n	+	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	+	n	−	−	n	59.9	+	n	+
Mrakia																																								
369.	v	+	+	−	v	v	v	−	+	n	−	+	+	v	n	n	n	n	n	n	n	n	n	−	−	+	+	v	n	n	v	−	n	n	n	8	54.4	+	−	+
Myxozyma																																								
370.	−	−	+	+	+	−	+	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	n	−	+	n	+	n	n	+	+	n	−	−	8	46.4	−	−	−
371.	+	+	+	−	+	v	v	−	−	n	−	+	v	−	n	n	n	n	n	n	n	n	n	n	−	×	n	−	n	n	+	+	n	−	−	8	39.5	−	−	−
372.	−	−	−	−	+	+	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	−	w	n	−	n	n	+	+	n	−	−	9	40.9	−	−	−
373.	−	+	×	−	+	−	+	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	n	−	w	n	−	n	n	+	+	n	−	−	8	50.3	−	−	−
374.	+	+	v	+	+	−	+	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	n	−	+	n	−	n	n	+	+	n	−	−	8	45.2	−	−	−
375.	+	+	+	v	×	×	−	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	n	−	+	n	−	n	n	+	+	+	+	n	8	42.4	−	−	−
376.	+	+	+	×	+	×	+	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	n	−	+	n	−	n	n	+	+	+	+	n	8	49.2	−	−	−
377.	+	+	+	+	v	−	−	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	n	−	+	n	−	n	n	+	+	n	−	−	8	44.6	−	−	−
Nadsonia																																								
378.	−	−	−	−	+	−	−	−	−	−	−	−	−	n	n	n	n	n	n	−	n	−	−	−	n	−	−	−	−	−	+	−	−	−	−	6	40.0	−	v	−
379.	+	−	−	+	+	−	−	−	−	−	v	−	−	n	n	n	n	n	n	+	n	+	v	−	n	−	−	−	−	−	+	+	−	−	−	6	41.5	−	+	−
380.	−	−	−	+	+	−	−	−	−	−	v	−	−	n	n	n	n	n	n	+	n	+	v	−	n	−	−	−	−	−	+	+	−	−	−	6	41.8	−	+	−
Oosporidium																																								
381.	−	−	n	+	+	−	−	n	+	n	−	n	n	n	n	n	−	n	n	n	n	n	n	−	n	+	n	n	n	n	+	+	v	−	−	n	n	−	−	v
Pachysolen																																								
382.	−	+	−	−	+	−	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	+	n	n	8	32.6	−	v	−
Pichia																																								
383.	+	×	v	−	+	+	−	+	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	v	n	n	+	+	+	+	n	n	46.0	−	+	−
384.	+	+	+	+	+	+	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	−	n	n	+	+	n	−	−	7	41.9	−	+	−
385.	×	+	×	+	+	+	−	−	+	n	−	v	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	7	43.6	−	v	+
386.	−	−	+	+	+	−	−	−	−	n	−	v	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	7	33.1	−	−	−
387.	−	−	+	+	+	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	7	33.2	−	−	−
388.	+	−	+	+	+	+	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	7	45.4	−	−	+
389.	+	×	−	−	+	×	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	n	33.0	−	+	−
390.	+	v	v	−	v	v	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	v	n	n	+	+	+	+	n	7	48.0	−	v	−
391.	+	+	v	+	+	+	−	−	+	n	+	−	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	−	n	n	+	+	n	v	n	7	36.6	−	v	−
392.	−	v	−	+	+	+	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	n	33.6	−	−	−
393.	−	−	−	+	+	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	+	+	n	7	36.2	−	+	−
394.	−	−	−	+	+	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	+	n	n	+	+	n	−	−	7	35.2	−	+	−
395.	v	+	+	+	+	+	−	−	+	n	−	v	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	7	41.5	−	v	+
396.	×	×	+	v	+	+	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	7	37.1	−	−	+
397.	+	+	+	+	+	+	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	7	42.3	−	+	−
398.	+	+	v	−	+	+	−	+	−	n	+	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	×	n	n	+	+	n	v	n	8	37.3	−	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

	Fermentation							Assimilation reactions and other characteristics																											
Species	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Pichia (*cont'd*)																																			
399. *P. cactophila*	v	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	×	–	–	–	–	+	–	–	+	+	–	–	–	–	–
400. *P. canadensis*	–	–	–	–	–	–	–	+	–	–	+	+	+	×	–	–	–	+	–	–	+	–	–	–	×	–	–	–	+	+	–	v	–	×	+
401. *P. capsulata*	+	–	–	–	–	–	+	+	–	–	–	+	+	+	v	–	–	v	–	+	+	v	v	+	v	+	+	+	+	+	+	+	–	+	+
402. *P. caribaea*	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	–	–	–	+	+	–	–	–	+	–
403. *P. castillae*	–	–	–	–	–	–	–	+	+	+	–	+	+	+	–	+	+	–	–	–	+	+	+	+	–	–	+	–	+	+	+	+	+	+	+
404. *P. chambardii*	–	–	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
405. *P. ciferrii*	+	×	+	v	–	w	–	+	+	–	+	+	×	+	–	–	+	+	–	+	×	×	–	+	×	–	–	–	+	+	+	+	–	+	+
406. *P. delftensis*	v	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	+	–	+	+
407. *P. deserticola*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
408. *P. dryadoides*	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	v	–	–	–	–	–	–	–	+	+	–	–	–	+	+
409. *P. euphorbiae*	+	–	w	–	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	–	+	+	–	–	+	–	–	–	+	+	–	–	–	+	+
410. *P. euphorbiiphila*	+	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	+	+
411. *P. fabianii*	+	–	+	w	–	w	–	+	–	–	+	+	+	+	–	–	+	+	–	+	+	–	–	–	–	–	–	–	+	+	–	–	–	+	+
412. *P. farinosa*	+	v	–	–	–	–	v	+	+	v	–	v	v	v	v	–	–	–	–	v	v	v	–	+	–	–	+	–	+	+	+	+	–	+	+
413. *P. fermentans*	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	+	+	–	–	–	–	–
414. *P. finlandica*	–	–	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	–	–	+	v	+	+	+	–	–	+	+	+	+	+	–	+	+
415. *P. fluxuum*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	v	–	v	–	+	+
416. *P. galeiformis*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–
417. *P. glucozyma*	+	–	–	–	–	–	×	+	–	–	–	–	+	+	–	–	–	–	–	–	×	–	–	+	+	–	–	×	+	+	+	+	–	+	+
418. *P. guilliermondii*	+	v	+	–	–	+	+	+	+	v	+	+	+	+	–	+	+	+	+	–	+	+	+	+	v	+	+	–	+	+	–	+	v	+	+
419. *P. hampshirensis*	+	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	–	+	–	–	–	+	–	–	–	+	+	–	×	–	v	+
420. *P. haplophila*	–	–	–	–	–	–	–	+	+	v	–	–	–	–	–	–	–	–	–	–	+	+	v	+	–	–	w	–	+	+	+	+	+	+	+
421. *P. heedii*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–
422. *P. heimii*	+	+	+	v	–	–	+	+	+	–	+	+	+	+	–	–	+	+	–	–	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+
423. *P. henricii*	–	–	–	–	–	–	–	+	–	–	–	–	+	v	–	–	–	–	–	–	+	–	–	+	+	–	–	w	+	+	+	+	–	+	+
424. *P. holstii*	+	v	–	–	–	–	+	+	+	+	+	+	+	+	–	–	–	+	–	+	+	+	+	+	+	+	+	–	+	+	v	+	v	+	+
425. *P. inositovora*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	–	+	–	–	+	–	+	–	–	–	+	–	+	+	–	+	–	+	+
426. *P. jadinii*	+	–	+	–	–	w	–	+	–	–	+	+	+	+	–	–	+	+	×	–	+	–	–	–	–	–	–	–	+	+	–	–	–	+	+
427. *P. japonica*	×	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	–	+	–	–	–	+	–	–	–	+	+	–	v	–	+	+
P. kluyveri																																			
428. var. *kluyveri*	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	+	+	–	+	+	–	–	–	–	–
429. var. *cephalocereana*	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–	–	–	–
430. var. *eremophila*	v	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	–	–	–	–	–
431. *P. kodamae*	+	–	–	–	–	–	–	+	–	v	–	–	+	–	–	–	–	–	–	–	+	v	–	+	–	–	–	w	w	+	+	+	–	+	+
432. *P. lynferdii*	+	–	+	–	–	w	–	+	+	–	+	+	+	+	–	–	+	+	+	–	–	–	–	+	–	–	–	–	+	+	+	+	–	+	+
433. *P. media*	–	–	–	–	–	–	–	+	+	+	–	+	+	+	–	–	–	–	–	v	+	+	+	+	–	v	+	–	+	+	+	+	v	+	+
434. *P. membranifaciens*	v	–	–	–	–	–	–	+	–	v	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	v	+	–	+	v	–	–	–	–	–
435. *P. methanolica*	+	–	–	–	–	–	+	+	+	v	v	–	+	+	–	–	–	–	–	–	+	+	×	+	–	–	–	+	+	+	+	+	+	+	+
436. *P. methylivora*	–	–	–	–	–	–	–	+	–	+	+	–	–	+	–	–	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
437. *P. mexicana*	+	×	–	–	–	–	+	+	+	–	+	+	+	+	+	v	+	+	–	–	+	+	v	+	+	+	+	–	+	+	+	+	v	+	+
438. *P. meyerae*	+	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	+	–	–	–	v	v
P. minuta																																			
439. var. *minuta*	×	–	–	–	–	–	w	+	–	–	–	–	+	+	–	–	–	–	–	–	+	–	v	+	v	–	–	+	×	+	–	+	–	+	+
440. var. *nonfermentans*	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	–	–	–	–	–	v	–	–	+	–	–	–	+	×	+	–	+	–	+	+
441. *P. mississippiensis*	+	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	–	+	+	v	–	v	–	–	–	+	+	–	–	–	+	+
442. *P. naganishii*	+	–	–	–	–	–	+	+	+	–	+	+	+	+	–	–	–	+	–	–	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+
443. *P. nakasei*	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
P. nakazawae																																			
444. var. *nakazawae*	+	+	–	w	–	–	+	+	+	+	+	+	+	+	–	–	–	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+
445. var. *akitaensis*	+	–	w	w	–	–	w	+	+	+	+	+	+	+	–	–	–	+	–	+	+	+	+	+	–	w	+	–	+	+	+	+	–	+	+
446. *P. norvegensis*	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	–	–	–	–	–

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Pichia *(cont'd)*																																								
399.	–	–	–	+	+	v	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	+	n	7	36.3	–	+	–
400.	v	+	+	+	+	+	–	–	v	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	+	n	7	40.3	–	v	v
401.	v	+	v	–	v	–	–	–	×	n	–	+	–	–	n	n	n	n	n	n	n	n	n	n	v	–	n	–	n	n	+	+	n	v	n	8	46.9	–	v	–
402.	–	–	+	+	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	v	n	n	34.2	–	–	–
403.	–	+	+	–	+	+	–	+	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	n	w	n	9	39.5	–	+	–
404.	–	+	–	+	+	w	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	7	33.8	–	+	–
405.	+	+	+	+	+	+	–	–	+	n	+	–	–	–	n	n	n	n	n	n	n	n	n	n	w	–	n	+	n	n	+	+	n	v	n	7	32.2	–	+	v
406.	–	–	–	–	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	n	–	–	7	33.2	–	+	–
407.	–	–	–	×	w	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	+	n	n	27.9	–	v	–
408.	–	–	+	+	+	+	–	–	+	n	+	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	v	n	n	31.0	–	–	–
409.	+	+	+	+	+	+	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	v	n	n	n	–	–	+
410.	+	+	+	+	+	+	–	–	+	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	v	–	n	–	n	n	+	+	+	+	n	n	47.3	–	–	–
411.	+	+	+	+	+	+	–	–	+	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	+	+	n	7	46.7	–	+	–
412.	v	v	+	–	v	+	–	+	–	n	v	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	v	n	n	+	+	+	+	n	9	42.7	–	+	–
413.	–	–	–	+	+	+	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	v	–	n	–	n	n	+	+	+	+	n	7	43.1	–	+	–
414.	–	–	v	–	v	–	–	–	+	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	v	n	n	+	+	n	–	–	7	45.4	–	–	–
415.	–	–	–	v	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	7	33.8	–	+	–
416.	–	–	–	w	+	–	–	–	–	n	+	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	+	+	n	7	35.6	–	+	–
417.	–	v	v	–	–	×	–	–	+	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	w	–	n	v	n	n	+	+	+	+	n	7	45.1	–	–	–
418.	+	+	v	v	+	v	–	+	–	n	–	+	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	+	+	n	9	44.4	–	v	–
419.	+	+	v	+	+	+	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	n	39.3	–	–	–
420.	–	–	v	–	–	–	–	+	–	n	–	+	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	v	n	n	+	+	n	–	–	9	41.0	–	+	–
421.	–	–	–	v	+	v	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	+	n	7	32.5	–	+	–
422.	+	+	v	–	+	+	–	+	–	n	+	+	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	n	–	–	n	39.7	–	+	–
423.	–	+	v	–	v	–	–	–	+	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	v	–	n	×	n	n	+	+	+	+	n	7	49.6	–	–	–
424.	+	+	v	–	+	+	–	–	+	n	–	v	–	–	n	n	n	n	n	n	n	n	n	n	×	–	n	v	n	n	+	+	n	v	n	8	37.1	–	v	+
425.	+	+	–	–	+	+	+	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	9	49.9	–	+	–
426.	+	+	+	+	+	+	–	–	+	n	+	v	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	+	n	7	45.5	–	v	–
427.	+	+	×	+	+	×	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	w	n	n	+	+	+	+	+	n	46.5	–	–	–
428.	–	–	–	×	×	v	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	v	n	n	+	+	n	v	n	7	30.4	–	+	–
429.	–	–	–	+	+	w	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	+	+	n	n	30.2	–	+	–
430.	–	–	–	+	+	×	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	v	n	n	+	+	+	+	n	n	30.3	–	+	–
431.	–	+	v	–	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	w	n	7	n	–	–	–
432.	+	+	+	+	+	+	–	–	+	n	+	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	n	–	–	n	39.3	–	+	–
433.	–	v	+	–	+	+	–	+	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	n	–	–	9	36.1	–	+	–
434.	–	–	–	v	v	v	–	–	–	n	v	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	v	n	n	+	+	n	v	n	7	44.3	–	+	–
435.	–	+	v	v	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	v	n	n	+	+	n	–	–	7/8	36.7	–	–	–
436.	–	–	+	+	–	+	–	–	+	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	n	n	7	35.8	–	–	–
437.	+	+	+	–	+	+	–	+	–	n	–	+	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	w	n	n	+	+	+	+	n	9	42.4	–	–	–
438.	v	+	+	+	+	+	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	+	n	7	48.7	–	–	v
439.	–	+	v	–	v	+	–	–	v	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	v	–	n	v	n	n	+	+	n	–	–	7	47.2	–	–	–
440.	–	+	v	–	+	×	–	–	+	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	w	n	n	+	+	+	+	n	7	45.2	–	–	–
441.	+	v	+	+	+	v	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	+	+	n	7	47.6	–	–	+
442.	w	+	–	+	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	w	n	n	+	+	+	+	n	7	46.1	–	–	–
443.	–	–	v	–	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	×	–	n	v	n	n	+	+	n	–	–	n	33.1	–	+	–
444.	+	×	+	×	+	+	–	+	–	n	–	+	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	n	–	–	9	39.4	–	+	–
445.	+	w	+	–	+	+	–	+	–	n	–	+	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	n	–	–	n	39.9	–	+	–
446.	–	+	–	w	+	w	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	+	+	+	+	n	7	37.5	–	+	–

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	N-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Pichia (*cont'd*)																																			
447. *P. ofunaensis*	w	w	–	–	–	–	w	+	+	+	v	v	+	+	+	+	v	v	–	–	+	+	–	+	+	+	+	–	+	+	v	+	+	+	+
448. *P. ohmeri*	+	×	+	v	–	+	v	+	+	+	+	+	+	+	–	–	+	–	v	–	–	–	–	v	–	+	+	–	+	+	–	v	–	+	+
449. *P. onychis*	+	–	+	–	–	w	–	+	–	–	+	+	+	+	–	–	+	+	v	–	+	v	v	–	–	–	–	–	+	+	–	–	–	+	+
450. *P. opuntiae*	–	–	–	–	–	–	–	+	–	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–	+	–
451. *P. pastoris*	+	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	v	–	–	–	+	–	–	+	+	+	–	–	–	+	+
452. *P. petersonii*	+	–	+	–	–	w	–	+	–	–	+	+	+	+	–	–	+	+	–	–	+	–	–	–	+	–	–	–	+	+	–	–	–	+	+
453. *P. philodendri*	–	–	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	–	–	+	v	–	+	–	–	–	+	+	+	+	+	–	+	+
454. *P. philogaea*	+	w	–	v	–	–	+	+	+	+	+	+	+	+	–	–	–	+	–	–	+	+	+	+	–	+	+	–	+	+	+	+	–	+	+
455. *P. pijperi*	+	–	–	–	–	–	–	+	–	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	+	+
456. *P. pini*	v	–	–	–	–	–	v	+	–	v	–	–	+	+	–	–	–	–	–	–	v	v	v	v	v	–	–	×	v	v	+	+	–	+	+
457. *P. populi*	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	+	–	–	–	+	+
458. *P. pseudocactophila*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	–	–	–	–	–
459. *P. quercuum*	w	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+
460. *P. rabaulensis*	+	–	+	–	–	w	w	+	–	–	+	+	+	+	–	–	+	+	–	–	+	+	–	–	v	–	–	–	+	+	–	+	–	+	+
461. *P. rhodanensis*	+	–	v	–	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	–	+	–	–	–	+	–	–	–	+	+	–	–	–	+	+
462. *P. salicaria*	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	+	–	–	–	+	+
463. *P. scolyti*	w	w	v	v	–	–	w	+	+	–	+	+	+	+	v	+	+	+	–	–	+	+	v	+	+	v	+	–	+	+	+	+	–	+	+
464. *P. segobiensis*	+	+	–	–	–	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	+	–	+	+	–	+	+	–	+	–	+	+
465. *P. silvicola*	+	×	–	–	–	–	–	+	+	v	v	v	+	v	–	–	–	v	–	–	+	+	–	v	+	–	–	–	+	+	v	+	–	v	+
466. *P. spartinae*	+	–	+	v	–	–	–	+	–	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	+	–	+	+	–	+	–	+	+
467. *P. stipitis*	+	w	–	+	–	–	+	+	+	v	+	+	+	+	v	–	–	+	–	+	+	v	+	+	+	+	+	–	+	+	+	+	–	+	+
468. *P. strasburgensis*	+	×	+	–	–	w	–	+	+	–	+	+	+	+	–	–	+	+	–	–	+	+	–	v	+	–	–	–	+	+	–	+	–	+	+
469. *P. subpelliculosa*	+	–	+	v	–	v	–	+	v	–	+	+	v	+	–	–	+	v	–	v	v	v	v	v	–	–	–	–	+	+	+	v	–	+	+
470. *P. sydowiorum*	+	×	+	v	–	w	+	+	+	–	+	+	+	+	–	+	+	+	–	v	v	+	–	+	+	–	–	–	+	+	+	+	–	+	+
471. *P. tannicola*	×	w	–	–	–	–	×	+	+	–	–	–	–	+	v	+	–	–	–	–	+	+	–	–	+	+	+	–	+	+	v	+	+	+	+
472. *P. thermotolerans*	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	v	–	–	–	+	+	–	–	–	+	v
473. *P. toletana*	w	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	–	v	–	–	+	–	–	–	v	–	×	–	+	+	–	–	–	+	+
474. *P. trehalophila*	+	–	–	–	–	–	v	+	–	+	–	–	–	+	–	–	–	–	–	–	+	+	v	+	–	–	–	+	+	+	+	+	–	+	+
475. *P. triangularis*	v	–	–	–	–	–	–	+	+	–	+	+	+	+	–	–	–	+	–	–	+	+	–	×	+	–	+	–	+	+	+	+	w	+	+
476. *P. veronae*	+	–	+	–	–	–	–	+	–	–	+	+	+	+	–	–	w	+	–	–	+	+	–	–	+	–	–	–	+	+	–	–	–	+	+
477. *P. wickerhamii*	+	–	–	–	–	–	–	+	–	–	+	+	+	v	–	–	–	+	–	–	+	v	–	–	+	–	–	–	+	+	–	+	–	+	+
478. *P. xylosa*	v	–	–	–	–	–	–	+	–	–	+	+	+	v	–	–	–	v	–	–	+	–	–	–	×	v	+	–	+	+	–	–	–	+	+
Protomyces																																			
479. *P. gravidus*	–	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	+	+	+	–	+	–	+	–	–	–	–	–	–	+	–	–	–	+	+
480. *P. inouyei*	–	–	–	–	–	–	–	+	v	–	+	+	+	+	–	–	+	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	v	+	+
481. *P. inundatus*	–	–	–	–	–	–	–	+	–	–	+	+	–	+	–	–	+	+	+	+	+	–	+	–	–	–	–	–	–	+	–	+	–	+	+
482. *P. lactucaedebilis*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	–	–	+	–	w	+	–	+	+	+	+
483. *P. macrosporus*	–	–	–	–	–	–	–	+	–	–	+	+	–	+	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	+	–
484. *P. pachydermus*	–	–	–	–	–	–	–	+	–	v	+	+	v	+	–	–	+	+	+	v	v	–	v	–	–	–	–	–	–	+	–	v	v	+	+
Prototheca																																			
485. *P. moriformis*	–	–	–	–	–	–	–	+	–	n	–	w	n	–	v	–	–	–	n	–	–	–	n	n	–	n	n	n	n	v	n	n	n	–	n
486. *P. stagnora*	–	–	–	–	–	–	–	+	+	n	–	w	n	–	v	–	–	–	n	–	–	–	n	n	–	n	n	n	n	v	n	n	n	–	n
487. *P. ulmea*	–	–	–	–	–	–	–	+	–	n	–	–	n	–	–	–	–	–	n	–	–	–	n	n	–	n	n	n	n	n	n	n	n	–	n
488. *P. wickerhamii*	–	–	–	–	–	–	–	+	+	–	–	–	–	+	v	–	–	–	–	–	–	–	–	–	–	n	–	–	+	+	–	n	n	–	n
P. zopfii																																			
489. var. *zopfii*	–	–	–	–	–	–	–	+	v	–	–	w	–	–	v	–	v	–	–	–	v	–	–	–	–	n	–	–	+	+	–	n	n	–	n
490. var. *hydrocarbonea*	–	–	–	–	–	–	–	+	v	–	–	w	–	–	v	–	v	–	–	–	v	–	–	–	–	n	–	–	+	+	–	n	n	–	n
491. var. *portoricensis*	–	–	–	–	–	–	–	+	v	–	–	w	–	–	v	–	v	–	–	–	v	–	–	–	–	n	–	–	+	+	–	n	n	–	n
Pseudozyma																																			
492. *P. antarctica*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	v	+	n	–	+	+	+	+	–	+	+
493. *P. aphidis*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	n	–	–	+	+	+	–	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Pichia (*cont'd*)																																								
447.	v	+	+	+	+	+	+	−	+	n	−	w	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	+	+	n	n	32.6	−	−	−
448.	+	+	v	v	+	+	−	+	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	v	n	n	+	+	+	+	n	9	44.5	−	+	−
449.	+	+	+	+	+	+	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	−	n	n	+	+	+	+	n	7	42.7	−	v	−
450.	−	+	−	+	+	×	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	7	33.2	−	−	−
451.	−	−	−	+	+	v	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	−	n	n	+	+	+	×	n	8	42.8	−	−	−
452.	+	+	+	+	+	+	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	7	44.9	−	+	v
453.	−	−	−	−	+	+	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	+	+	n	7	49.7	−	−	−
454.	+	−	+	−	+	+	−	+	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	v	n	n	+	+	+	w	n	9	42.0	−	+	−
455.	−	+	−	+	+	v	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	v	n	7	42.5	−	+	−
456.	−	+	v	−	v	v	−	−	−	n	v	v	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	v	n	n	+	+	n	v	n	7	43.0	−	−	−
457.	−	+	+	+	+	−	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	7	44.0	−	−	−
458.	−	−	−	+	+	v	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	v	n	n	+	+	+	+	n	n	36.6	−	+	−
459.	−	+	−	+	+	×	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	×	n	7	42.7	−	−	−
460.	+	v	+	+	+	+	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	+	+	n	7	44.2	−	+	−
461.	v	v	+	+	+	+	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	7	51.9	−	v	v
462.	−	+	+	+	+	+	−	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	7	37.8	−	+	−
463.	+	+	+	−	+	+	−	+	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	v	n	n	+	+	n	v	n	9	40.1	−	v	v
464.	+	+	+	−	+	+	−	+	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	v	n	n	+	+	n	−	−	9	42.0	−	v	−
465.	+	+	+	v	+	v	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	v	n	7	36.5	−	v	v
466.	+	+	v	v	+	+	−	+	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	+	+	n	n	41.1	−	v	−
467.	+	+	v	+	+	+	−	+	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	−	n	n	+	+	n	v	n	9	43.4	−	v	−
468.	+	+	+	+	+	+	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	n	v	n	7	40.6	−	−	−
469.	+	+	+	+	+	+	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	v	n	n	+	+	n	v	n	7	34.0	−	v	v
470.	+	+	+	+	+	+	−	−	+	n	+	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	v	n	n	+	+	n	−	−	n	36.2	−	+	−
471.	−	−	v	+	+	×	v	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	n	43.0	−	+	−
472.	−	+	−	+	+	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	+	+	n	n	32.9	−	v	−
473.	v	+	v	×	+	w	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	7	39.6	−	v	−
474.	−	−	−	−	+	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	−	n	n	+	+	n	−	−	7	37.6	−	−	−
475.	w	−	+	−	+	v	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	n	−	−	9	36.6	−	−	−
476.	+	w	+	+	+	w	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	7	49.5	−	+	−
477.	+	+	+	+	+	+	−	−	−	n	−	×	+	−	n	n	n	n	n	n	n	n	n	n	×	−	n	v	n	n	+	+	+	+	n	7	46.1	−	+	v
478.	v	+	v	×	+	v	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	−	n	−	n	n	+	+	n	−	−	7	39.9	−	v	−
Protomyces																																								
479.	−	+	−	+	+	+	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	w	n	−	−	−	+	+	n	−	−	n	n	−	v	v
480.	+	w	−	+	+	v	−	v	v	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	w	n	−	−	−	+	n	n	−	−	10	52.0	−	v	v
481.	v	−	−	v	×	×	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	v	n	v	−	−	+	+	n	−	−	n	n	−	−	v
482.	+	−	−	+	+	+	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	v	n	w	n	n	+	+	n	−	−	10	52.0	−	−	v
483.	−	−	−	−	+	+	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	w	n	−	−	−	+	n	n	−	−	n	n	−	−	v
484.	v	−	−	v	v	w	−	−	v	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	−	+	n	w	−	−	+	n	n	−	−	10	52.4	−	−	v
Prototheca																																								
485.	n	n	n	n	n	n	−	−	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	n	n	+	+	v	v	n	n	n	n	n	−
486.	n	n	n	n	+	n	−	−	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	n	n	+	+	n	n	n	n	n	n	n	−
487.	n	n	n	n	n	n	−	−	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	n	n	+	+	n	n	n	n	n	n	n	−
488.	−	−	−	+	n	n	−	−	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	n	n	+	+	v	v	n	n	n	n	n	−
489.	−	−	−	+	−	−	−	−	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	n	n	+	+	v	v	n	n	n	n	n	−
490.	−	−	−	+	−	−	−	+	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	n	n	+	+	v	v	n	n	n	n	n	−
491.	−	−	−	+	−	−	−	−	−	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	n	n	n	n	+	+	v	v	n	n	n	n	n	−
Pseudozyma																																								
492.	+	+	+	+	+	+	+	n	+	+	−	+	n	−	n	n	n	n	n	n	n	n	n	−	n	−	+	n	n	n	+	+	n	v	n	10	61.4	+	−	+
493.	+	+	+	+	+	+	+	n	+	+	−	+	n	+	n	n	n	n	n	n	n	n	n	−	n	−	+	n	n	n	+	+	+	+	n	10	61.5	+	−	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Pseudozyma (*cont'd*)																																			
494. *P. flocculosa*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	–	+	n	–	–	+	+	+	–	+	+
495. *P. fusiformata*	–	–	–	–	–	–	–	+	–	+	+	+	+	+	–	v	+	+	–	–	+	+	+	+	–	v	n	–	+	+	+	+	–	+	+
496. *P. prolifica*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	+	n	–	+	+	+	+	–	+	+
497. *P. rugulosa*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	n	w	+	+	+	+	–	+	+
498. *P. tsukubaënsis*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	–	+	n	–	+	+	+	–	–	–	–
Reniforma																																			
499. *R. strues*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Rhodosporidium																																			
500. *R. babjevae*	–	–	–	–	–	–	–	+	+	+	+	+	×	+	–	–	+	+	v	w	v	–	+	w	+	–	n	n	+	+	–	v	–	+	+
501. *R. dacryoideum*	–	–	–	–	–	–	–	+	+	v	v	v	v	+	–	–	–	v	–	–	–	–	–	–	v	–	–	–	v	+	–	–	–	v	×
502. *R. diobovatum*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	w	+	+	+	+	–	–	–	–	+	+	–	+	v	v	+
503. *R. fluviale*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	–	–	+	+	+	+	–	–	–	–	+	+	–	+	+	+	+
504. *R. kratochvilovae*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	w	+	–	–	–	+	+	–	+	–	+	+
505. *R. lusitaniae*	–	–	–	–	–	–	–	+	+	+	–	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	+	+	–	+	+	+	+
506. *R. malvinellum*	–	–	–	–	–	–	–	+	+	v	+	+	v	+	–	–	+	–	–	–	+	v	v	–	+	–	–	–	v	+	–	v	v	+	v
507. *R. paludigenum*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	–	–	–	+	+	+	+	+	–	–	–	+	+	–	+	+	+	+
508. *R. sphaerocarpum*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	+	–	–	–	–	+	+	–	+	–	v	v
509. *R. toruloides*	–	–	–	–	–	–	–	+	+	v	+	+	v	+	–	–	+	+	+	–	+	+	+	+	–	–	–	–	+	+	–	+	–	+	+
Rhodotorula																																			
510. *R. acheniorum*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	w	+	+	+	–	–	+	+	+	+	–	+	+	–	+	+	+	w	–	+	+
511. *R. acuta*	–	–	–	–	–	–	–	+	v	+	+	–	+	+	+	–	+	–	–	–	+	+	+	+	–	–	+	–	+	+	+	+	–	+	+
512. *R. araucariae*	–	–	–	–	–	–	–	+	+	+	–	–	–	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–	+	+	–	+	–	+	+
513. *R. armeniaca*	–	–	–	–	–	–	–	+	+	v	–	–	–	–	–	–	–	–	–	–	+	+	+	+	v	–	–	–	–	+	–	v	v	+	+
514. *R. aurantiaca*	–	–	–	–	–	–	–	+	+	v	+	+	v	v	–	–	–	+	–	–	+	v	v	v	–	–	–	–	v	+	–	v	v	+	+
515. *R. auriculariae*	–	–	–	–	–	–	–	+	–	–	+	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	w	–	–	–	–	+	+
516. *R. bacarum*	–	–	–	–	–	–	–	+	–	v	+	+	+	+	–	–	+	+	–	+	+	+	+	+	–	–	–	–	–	+	+	+	–	+	+
517. *R. bogoriensis*	–	–	–	–	–	–	–	+	+	+	–	+	+	+	–	–	–	+	–	+	+	+	v	+	–	+	+	–	+	+	–	+	–	+	+
518. *R. buffonii*	–	–	–	–	–	–	–	+	–	+	–	+	+	+	–	–	–	+	–	v	+	+	–	–	–	+	+	–	+	+	–	+	–	+	+
519. *R. diffluens*	–	–	–	–	–	–	–	+	+	+	+	+	–	+	–	–	–	+	–	–	v	–	–	–	–	+	+	–	+	+	+	+	–	+	+
520. *R. ferulica*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+	+	–	+	+	+	–	–	v	v
521. *R. foliorum*	–	–	–	–	–	–	–	+	–	–	–	–	+	+	+	–	–	–	–	–	+	v	v	v	–	+	+	–	+	+	–	v	–	+	+
522. *R. fragaria*	–	–	–	–	–	–	–	+	v	+	+	+	+	+	+	–	+	+	–	–	+	+	+	v	–	+	+	–	+	v	–	v	–	+	+
523. *R. fujisanensis*	–	–	–	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	+	+	–	+	+	+	+
524. *R. futronensis*	–	–	–	–	–	–	–	+	+	+	–	–	+	+	–	–	–	–	–	–	+	+	+	–	–	–	–	–	+	+	–	+	+	+	+
525. *R. glutinis*	–	–	–	–	–	–	–	+	v	v	+	+	v	+	–	–	v	+	–	–	v	v	v	v	v	–	–	–	v	v	–	v	v	v	v
526. *R. graminis*	–	–	–	–	–	–	–	+	+	v	+	v	v	+	–	–	+	–	–	–	×	v	v	v	v	–	–	–	v	v	–	v	v	v	v
527. *R. hinnulea*	–	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	+	+	–	–	–	+	–	–	–	–	–	–	v	+	+	–	–	+	+
528. *R. hordea*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	–	+	–	v	v	–	v	+	–	–	–	–	–	+	+	+	–	+	+
529. *R. hylophila*	–	–	–	–	–	–	–	+	–	v	–	–	–	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	+	+	–	–	–	+	+
530. *R. ingeniosa*	–	–	–	–	–	–	–	+	w	+	+	+	+	+	+	–	+	+	–	+	+	–	–	+	–	+	+	–	–	+	–	–	–	+	+
531. *R. javanica*	–	–	–	–	–	–	–	+	+	+	–	+	+	+	–	+	+	–	–	+	+	+	+	+	–	–	–	–	+	+	–	+	+	+	+
532. *R. lactosa*	–	–	–	–	–	–	–	+	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+	–	+	–	+	–	+	+	–	+	–	+	+
533. *R. lignophila*	–	–	–	–	–	–	–	+	–	–	–	–	+	+	+	–	–	–	–	–	+	–	–	–	–	–	+	–	+	+	–	–	–	+	+
534. *R. marina*	–	–	–	–	–	–	–	+	+	w	+	v	+	+	v	–	w	+	–	w	+	+	+	w	+	–	–	–	–	+	–	w	+	+	+
535. *R. minuta*	–	–	–	–	–	–	–	+	v	v	+	–	v	+	v	–	–	+	–	–	+	+	+	v	–	–	+	–	v	+	–	v	–	v	v
536. *R. mucilaginosa*	–	–	–	–	–	–	–	+	v	v	+	v	v	+	–	–	+	v	–	–	+	v	v	v	v	v	–	–	v	v	–	v	v	v	v
537. *R. muscorum*	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+	–	–	+	–	+	+	–	+	+	–	+	+	–	+	+	+	+
538. *R. nothofagi*	–	–	–	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	+	+	–	+	+	+	+
539. *R. philyla*	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	v	–	–	–	–	+	+	–	+	–	–	+	–	+	+
540. *R. phylloplana*	–	–	–	–	–	–	–	+	–	–	+	+	–	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–	+	+	+	–	–	+	+
541. *R. pilati*	–	–	–	–	–	–	–	+	–	–	+	+	+	+	v	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Pseudozyma (*cont'd*)																																								
494.	+	+	+	+	+	+	+	n	+	+	+	+	n	−	n	n	n	n	n	n	n	n	n	−	n	−	+	n	n	n	+	+	n	−	−	n	n	+	−	+
495.	+	v	+	+	+	+	+	n	+	+	+	+	n	−	n	n	n	n	n	n	n	n	n	−	n	−	+	n	n	n	+	+	n	−	−	n	58.1	+	−	+
496.	+	−	+	+	+	−	+	n	+	+	+	+	n	−	n	n	n	n	n	n	n	n	n	−	n	−	+	n	n	n	+	+	n	−	−	n	n	+	−	+
497.	+	+	+	+	+	+	+	n	+	+	w	+	n	+	n	n	n	n	n	n	n	n	n	−	n	−	+	n	n	n	+	+	+	+	n	n	n	+	−	+
498.	+	−	−	+	+	+	+	n	+	+	+	+	n	−	n	n	n	n	n	n	n	n	n	−	+	−	+	n	n	n	+	+	n	−	−	n	53.3	+	−	+
Reniforma																																								
499.	−	−	−	−	−	+	−	+	−	n	×	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	+	n	n	n	n	+	n	−	−	n	n	+	+	−
Rhodosporidium																																								
500.	v	+	n	+	+	+	−	n	+	+	+	+	−	n	w	−	+	n	n	+	−	+	+	+	n	−	+	n	n	n	+	+	−	n	n	10	66.2	+	v	−
501.	−	−	+	+	+	+	−	−	−	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	−	n	10	58.3	+	−	v
502.	+	+	+	−	×	×	−	+	+	n	+	n	n	+	n	n	n	n	n	n	n	n	n	−	+	−	+	−	n	n	+	+	+	−	n	10	66.1	+	−	v
503.	+	+	+	w	+	w	−	−	+	n	+	−	n	−	n	n	n	n	n	n	n	n	n	−	w	−	+	−	n	n	+	+	n	−	n	n	61.4	+	−	+
504.	+	+	+	−	+	w	−	−	+	n	+	n	n	−	n	n	n	n	n	n	n	n	n	n	+	−	+	−	n	n	+	+	n	−	n	10	64.8	+	−	+
505.	−	+	+	−	×	−	−	−	+	n	+	n	n	−	n	n	+	n	n	−	−	n	+	−	+	−	+	−	−	−	+	+	n	−	n	9	62.0	+	−	+
506.	−	+	−	+	+	−	−	−	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	−	n	n	n	9	50.5	+	−	−
507.	+	+	+	−	w	w	−	−	+	n	+	n	n	+	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	−	n	10	65.1	+	−	+
508.	+	+	v	−	−	−	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n	n	n	−	+	−	+	−	n	n	+	+	−	n	n	10	64.6	+	−	v
509.	+	+	+	v	w	w	−	+	+	n	+	−	−	−	n	n	n	n	n	n	n	n	n	−	+	−	+	v	n	n	+	+	+	w	w	9	60.7	+	−	v
Rhodotorula																																								
510.	+	−	−	+	+	+	−	+	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	w	−	+	−	n	n	+	+	−	−	n	10	53.9	+	−	−
511.	−	+	+	w	+	+	−	−	−	n	v	n	n	−	n	n	n	n	n	n	n	n	n	+	+	−	+	−	n	n	+	+	+	+	n	9	53.7	+	+	−
512.	−	−	+	w	−	−	−	−	+	n	+	+	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	−	n	10	64.6	+	−	−
513.	−	−	−	−	w	−	−	−	−	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	n	n	n	n	+	−	−
514.	−	+	+	v	+	v	−	−	+	n	−	+	v	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	−	n	10	57.1	+	−	−
515.	−	−	+	−	+	−	−	−	−	n	−	n	n	+	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	−	n	n	54.4	+	−	−
516.	+	+	−	−	+	+	−	−	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	−	n	n	52.4	+	−	−
517.	−	+	+	−	×	×	−	−	−	n	−	n	n	w	n	n	n	n	n	n	n	n	n	−	+	−	+	−	n	n	+	+	−	−	n	10	57.3	+	−	−
518.	−	v	+	−	v	v	−	−	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	+	−	+	−	n	n	+	+	−	−	n	n	50.8	+	+	−
519.	−	+	+	−	+	+	−	−	+	n	+	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	−	n	10	60.5	+	−	−
520.	+	−	+	w	+	+	−	−	+	n	−	+	n	+	n	n	+	n	n	v	−	+	v	−	−	−	+	−	+	−	+	+	−	−	n	10	66.1	+	−	−
521.	−	−	+	+	+	+	−	−	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	n	n	10	56.0	+	−	−
522.	+	+	+	v	+	+	−	−	+	n	+	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	n	n	n	56.6	+	−	−
523.	−	+	+	−	−	w	−	−	−	n	−	n	n	−	n	n	n	n	n	n	n	n	n	+	+	−	+	−	n	n	+	+	−	n	n	9	63.4	+	+	−
524.	−	+	+	−	+	w	−	−	−	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	−	n	n	n	+	−	−
525.	v	×	+	v	+	v	−	+	+	n	v	v	−	−	n	n	n	n	n	n	n	n	n	−	w	−	+	−	n	n	+	+	+	+	n	10	63.9	+	−	−
526.	v	v	+	−	+	v	−	−	+	n	+	+	−	+	n	n	n	n	n	n	n	n	n	−	+	−	+	−	n	n	+	+	n	−	n	10	68.5	+	−	−
527.	+	−	−	−	w	w	+	−	+	n	+	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	−	n	n	n	+	−	−
528.	v	+	−	+	+	+	−	+	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	n	n	n	46.9	+	−	−
529.	−	−	−	w	+	−	−	−	−	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	−	n	n	48.3	+	−	−
530.	+	+	+	w	w	w	−	+	+	n	+	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	v	n	10	55.6	+	−	−
531.	−	+	+	−	+	+	−	−	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	+	−	+	−	n	n	+	+	−	n	n	9	59.0	+	−	−
532.	w	+	+	w	+	+	−	−	+	n	−	+	+	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	−	n	9	57.3	+	−	−
533.	−	−	+	+	w	+	−	−	−	n	−	n	n	+	n	n	n	n	n	n	n	n	n	+	−	−	+	−	n	n	+	+	−	n	n	n	n	+	−	−
534.	w	w	w	w	+	+	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	n	n	10	53.9	+	−	−
535.	−	v	+	v	+	−	−	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	v	n	10	50.8	+	−	−
536.	v	v	+	v	+	v	−	−	−	n	v	−	−	−	n	n	n	n	n	n	n	n	n	−	v	−	+	−	n	n	+	+	+	+	n	10	60.6	+	−	−
537.	+	+	+	−	+	+	−	−	+	n	+	n	n	+	n	n	n	n	n	n	n	n	n	−	+	−	+	−	n	n	+	+	−	n	n	10	n	+	−	−
538.	−	+	+	−	w	w	−	−	−	n	+	n	n	−	n	n	n	n	n	n	n	n	n	+	+	−	+	−	n	n	+	+	n	−	n	n	n	+	−	−
539.	−	−	+	−	+	+	−	−	−	n	+	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	n	−	n	10	62.9	+	−	−
540.	+	−	−	−	+	−	+	−	+	n	+	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	n	n	n	n	+	−	−
541.	−	−	+	−	−	−	−	−	+	n	−	n	n	−	n	n	n	n	n	n	n	n	n	−	−	−	+	−	n	n	+	+	−	n	n	n	n	+	−	−

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Rhodotorula (*cont'd*)																																			
542. *R. pustula*	−	−	−	−	−	−	−	+	v	+	−	−	+	+	−	−	−	−	−	−	+	+	+	+	−	+	+	−	+	+	−	+	−	+	+
543. *R. sonckii*	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	−	−	−	+	v	−	−	−	−	−	−	−	+	−	−	−	+	+
Saccharomyces																																			
544. *S. barnettii*	+	+	+	−	−	+	+	+	+	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
545. *S. bayanus*	+	v	+	+	−	+	−	+	v	−	+	+	−	+	−	v	+	v	−	−	−	−	−	−	−	−	−	−	+	v	−	−	−	v	−
546. *S. castellii*	+	+	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	−	−	−	−	−	−
547. *S. cerevisiae*	+	v	+	v	−	+	−	+	v	−	+	+	−	+	−	v	+	v	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
548. *S. dairenensis*	+	+	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	v	−	−	−	−	v	v	−	−	−	−	−
549. *S. exiguus*	+	+	+	−	−	+	+	+	+	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
550. *S. kluyveri*	+	+	+	−	−	+	−	+	+	v	+	+	−	+	−	+	+	v	−	−	−	−	−	−	−	−	−	−	+	v	−	−	−	+	+
551. *S. paradoxus*	+	+	+	v	−	+	−	+	+	−	+	+	−	+	−	−	+	v	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−
552. *S. pastorianus*	+	v	v	+	−	+	−	+	+	−	+	+	−	+	−	−	+	v	−	−	−	−	−	−	−	−	−	−	+	v	−	−	−	−	−
553. *S. rosinii*	+	+	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
554. *S. servazzii*	+	+	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−
555. *S. spencerorum*	+	+	+	−	−	−	+	+	+	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
556. *S. transvaalensis*	+	+	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	v	−	−	−	−	−
557. *S. unisporus*	+	+	−	−	−	−	−	+	+	−	−	−	−	v	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Saccharomycodes																																			
558. *S. ludwigii*	+	−	+	−	−	+	−	+	−	−	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×	+	−	−	−	−	−
Saccharomycopsis																																			
559. *S. capsularis*	+	−	−	×	−	−	−	+	−	−	−	+	+	v	−	−	−	−	−	+	−	−	v	v	−	−	−	−	+	+	+	×	−	+	+
560. *S. crataegensis*	v	−	−	−	−	−	−	+	−	+	−	−	−	v	−	−	−	−	−	−	+	v	v	+	−	×	v	−	+	+	−	v	−	+	+
561. *S. fermentans*	+	−	−	−	−	−	−	+	−	v	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−
562. *S. fibuligera*	×	−	×	×	−	v	−	+	−	−	+	+	+	v	−	−	v	v	−	+	−	−	−	−	−	−	−	−	+	+	v	v	−	v	v
563. *S. javanensis*	−	−	−	−	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−	v	v	−	−	−	−	−	+	−	+	+	−	−	−	−	−
564. *S. malanga*	w	−	−	w	−	−	−	+	−	−	−	+	+	v	−	−	−	−	−	+	v	−	v	+	−	−	−	−	+	+	v	+	−	+	+
565. *S. schoenii*	−	−	−	−	−	−	−	+	−	v	v	−	−	+	−	−	−	−	−	v	v	−	−	−	−	−	−	−	+	+	−	−	−	−	−
566. *S. selenospora*	×	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	v	+	+	−	−	−	−	−	−	+	v	−	+	−	−	−
567. *S. synnaedendra*	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	+	v	−	+	+	+	−	−	−	+	+	+	+	−	+	+
568. *S. vini*	+	−	v	−	−	v	−	+	−	+	v	v	−	v	−	−	v	−	−	−	−	−	−	−	−	v	−	−	×	+	−	v	−	v	+
Saitoella																																			
569. *S. complicata*	−	−	−	−	−	−	−	+	×	×	+	+	+	+	−	+	+	+	+	−	+	−	+	v	−	n	n	−	+	+	−	+	−	+	+
Saturnispora																																			
570. *S. ahearnii*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	+
571. *S. dispora*	+	−	−	−	−	−	v	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	+
572. *S. saitoi*	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	v	−	+	+
573. *S. zaruensis*	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	+
Schizoblastosporion																																			
574. *S. starkeyi-henricii*	−	−	−	−	−	−	−	+	v	v	−	−	−	v	−	−	−	−	−	−	−	−	−	v	−	−	n	−	−	v	−	v	−	v	+
Schizosaccharomyces																																			
575. *S. japonicus*	+	−	+	v	−	+	−	+	−	−	+	v	−	−	−	−	+	−	v	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
576. *S. octosporus*	+	−	−	v	−	−	−	+	−	−	−	v	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−
577. *S. pombe*	+	−	+	+	−	×	−	+	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sirobasidium																																			
578. *S. intermedium*	−	−	−	−	−	−	−	+	v	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	−	n	−	+	+	+	+	+	+	+
579. *S. magnum*	−	−	−	−	−	−	−	+	+	+	−	v	+	+	+	−	−	+	−	−	+	+	+	+	+	−	n	−	+	+	+	+	+	+	+
Sporidiobolus																																			
580. *S. johnsonii*	−	−	−	−	−	−	−	+	v	+	+	+	+	+	−	−	−	+	−	v	w	v	+	+	−	−	−	−	+	+	−	+	−	+	+
581. *S. pararoseus*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	+	−	v	v	−	w	v	v	−	−	−	+	+	−	v	−	+	v
582. *S. ruineniae*	−	−	−	−	−	−	−	+	+	+	+	v	+	+	−	−	+	−	−	−	+	+	+	+	v	−	−	−	+	+	−	+	+	+	+
583. *S. salmonicolor*	−	−	−	−	−	−	−	+	v	v	+	v	v	+	−	−	v	v	−	v	+	v	v	+	−	−	−	−	+	v	−	+	−	+	+

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Rhodotorula *(cont'd)*																																								
542.	–	+	+	–	+	+	–	–	+	n	–	n	n	–	n	n	n	n	n	n	n	n	n	–	–	–	+	–	n	n	+	+	–	n	n	n	51.0	+	–	–
543.	–	–	+	–	+	–	–	–	+	n	–	+	–	–	n	n	n	n	n	n	n	n	n	–	–	–	v	–	n	n	+	+	–	n	n	n	44.1	+	–	–
Saccharomyces																																								
544.	–	–	+	+	+	–	–	–	–	–	–	n	n	n	n	n	–	n	n	–	n	–	–	n	+	–	n	–	–	–	+	+	–	–	–	n	33.4	–	–	–
545.	v	–	v	v	–	–	–	–	–	–	+	n	n	n	n	n	n	n	n	–	n	–	–	n	n	–	n	–	–	–	+	+	v	–	–	6	41.8	–	–	–
546.	–	–	+	–	v	–	–	–	–	–	–	n	n	n	n	n	n	n	n	–	n	–	–	n	–	–	n	–	–	–	+	+	+	v	–	6	37.9	–	–	–
547.	v	–	v	v	v	–	–	–	–	–	–	n	n	n	n	n	n	n	n	–	n	–	–	n	n	–	n	–	–	–	+	+	v	v	v	6	40.4	–	–	–
548.	–	–	v	–	+	–	–	–	–	–	–	n	n	n	n	n	n	n	n	–	n	–	–	n	–	–	n	–	–	–	+	+	+	v	–	6	36.7	–	–	–
549.	–	–	–	–	+	v	–	–	–	–	–	n	n	n	n	n	n	n	n	–	n	–	–	n	v	–	n	–	v	v	+	+	v	–	–	6	35.5	–	–	–
550.	+	–	–	+	–	–	–	–	–	–	–	n	n	n	n	n	n	n	n	+	n	+	+	n	n	–	n	–	–	–	+	+	+	+	v	n	41.9	–	–	–
551.	+	–	v	+	–	–	–	–	–	–	–	n	n	n	n	n	n	n	n	–	n	–	–	n	n	–	n	–	–	–	+	+	+	+	v	n	42.6	–	–	–
552.	v	–	v	v	–	–	–	–	–	–	–	n	n	n	n	n	n	n	n	–	n	–	–	n	n	–	n	–	–	–	+	+	v	–	–	6	41.1	–	–	–
553.	–	–	–	–	–	–	–	–	–	–	–	n	n	n	n	n	–	n	n	–	n	–	–	n	–	–	n	–	–	–	+	+	–	–	–	n	n	–	–	–
554.	–	–	–	–	–	–	–	–	–	–	–	n	n	n	n	n	n	n	n	–	n	–	–	n	n	–	n	–	+	+	+	+	+	–	–	n	36.6	–	–	–
555.	–	–	+	+	+	–	–	–	–	–	–	n	n	n	n	n	–	n	n	+	n	+	+	n	–	–	n	–	–	–	+	+	+	+	–	6	34.8	–	–	–
556.	–	–	+	–	–	–	–	–	–	–	–	n	n	n	n	n	–	n	n	v	n	v	–	n	–	–	n	–	–	–	+	+	+	+	–	6	33.5	–	–	–
557.	–	–	–	–	+	–	–	–	–	–	–	n	n	n	n	n	n	n	n	+	n	+	+	n	n	–	n	–	+	+	+	+	v	v	–	6	35.6	–	–	–
Saccharomycodes																																								
558.	–	+	–	+	–	–	–	–	–	–	–	–	–	n	n	n	n	n	n	+	n	+	+	–	–	–	–	w	–	–	+	+	n	–	–	6	38.3	–	–	–
Saccharomycopsis																																								
559.	v	v	+	–	+	v	–	–	–	n	–	+	+	–	n	n	n	n	n	n	n	n	n	n	×	–	n	w	n	n	+	+	n	–	–	8	43.4	–	v	+
560.	–	–	+	×	×	–	–	–	–	n	–	–	+	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	n	39.1	–	+	+
561.	–	–	–	–	v	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	8	37.0	–	–	+
562.	+	+	×	×	×	v	×	–	–	n	–	–	v	–	n	n	n	n	n	n	n	n	n	n	v	–	n	×	n	n	+	+	+	×	n	8	39.3	–	v	+
563.	–	–	–	–	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	8	31.3	–	–	+
564.	–	+	+	–	+	–	–	–	–	n	–	+	+	–	n	n	n	n	n	n	n	n	n	n	×	–	n	+	n	n	+	+	+	+	n	n	n	–	v	+
565.	–	–	–	–	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	8	34.1	–	–	+
566.	–	–	v	+	+	–	–	–	–	n	–	–	+	–	n	n	n	n	n	n	n	n	n	n	–	–	n	v	n	n	+	+	n	–	–	8	30.2	–	–	+
567.	–	–	+	+	×	v	+	–	–	n	–	–	+	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	+	+	n	–	–	8	42.0	–	v	+
568.	v	v	–	–	v	–	–	–	–	n	–	×	–	–	n	n	n	n	n	n	n	n	n	n	v	–	n	v	n	n	+	+	n	–	–	8	n	–	+	+
Saitoella																																								
569.	n	+	n	+	+	+	–	n	+	n	+	n	n	n	n	n	n	n	n	n	n	n	n	–	n	–	+	n	n	n	+	+	+	–	–	10	51.7	–	–	–
Saturnispora																																								
570.	–	–	–	+	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	w	n	n	n	+	n	–	n	n	34.9	–	+	–
571.	–	–	–	v	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	n	+	n	–	n	7	38.0	–	–	–
572.	–	–	–	+	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	+	n	n	n	+	n	–	n	7	33.3	–	+	–
573.	–	–	–	+	+	–	–	–	–	n	–	–	–	–	n	n	n	n	n	n	n	n	n	n	–	–	n	–	n	n	n	+	n	–	n	7	39.5	–	–	–
Schizoblastosporion																																								
574.	–	–	v	–	+	–	–	n	–	–	–	v	n	n	n	–	–	–	–	v	–	+	+	–	n	–	–	n	–	–	+	+	–	–	–	n	41.6	–	–	–
Schizosaccharomyces																																								
575.	–	–	–	–	–	–	–	–	–	n	–	n	n	n	n	n	n	n	n	–	n	–	–	n	n	n	n	n	–	–	+	+	+	+	n	–	34.3	–	–	+
576.	–	–	–	–	–	–	–	–	–	n	–	n	n	n	n	n	n	n	n	–	n	–	–	n	n	n	n	n	–	–	+	+	–	–	n	9	39.2	–	–	–
577.	v	–	+	–	–	–	–	–	–	n	–	n	n	n	n	n	n	n	n	–	n	–	–	n	n	n	n	n	–	–	+	+	n	–	n	10	38.2	–	–	–
Sirobasidium																																								
578.	+	+	+	+	+	+	+	n	–	–	+	+	n	n	n	n	n	n	n	n	n	n	n	–	n	+	+	n	n	n	+	+	n	–	–	n	n	+	–	v
579.	+	+	+	+	+	+	+	n	–	–	–	+	n	n	n	n	n	n	n	n	n	n	n	–	n	+	+	n	n	n	+	+	n	–	–	n	n	+	n	v
Sporidiobolus																																								
580.	+	×	+	v	+	v	–	+	+	n	+	–	–	–	n	n	+	n	n	n	n	n	n	–	+	–	+	v	n	n	+	+	+	v	–	10	63.0	+	+	+
581.	+	+	+	v	v	v	–	–	–	n	+	–	n	–	n	n	n	n	n	n	n	n	n	–	–	–	+	–	n	n	+	+	n	–	n	10	52.5	+	+	+
582.	–	+	+	–	v	w	–	–	+	n	+	–	–	+	n	n	n	n	n	n	n	n	n	v	+	–	+	–	n	n	+	+	n	–	n	10	64.7	+	w	+
583.	v	v	+	+	v	–	–	–	+	n	+	n	n	+	n	n	n	n	n	n	n	n	n	–	–	–	+	–	n	n	+	+	v	v	n	10	63.5	+	+	v

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																											
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Sporobolomyces																																			
584. *S. alborubescens*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	v	w	−	+	+	+	v	−	−	n	−	+	+	−	+	−	+	+
585. *S. elongatus*	−	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−	−	+	−	−	+	+	+	−	+	−	n	−	−	+	−	−	−	+	+
586. *S. falcatus*	−	−	−	−	−	−	−	+	w	−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	w	n	−	+	+	−	−	−	+	+
587. *S. foliicola*	−	−	−	−	−	−	−	+	+	+	+	−	w	+	−	−	−	+	−	−	+	+	+	+	−	−	n	−	−	+	−	+	−	+	+
588. *S. gracilis*	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	−	−	−	−	+	−	v	+	−	−	n	−	−	+	−	+	−	+	v
589. *S. griseoflavus*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	−	−	−	+	−	+	+	−	+	−	−	−	n	−	+	+	−	+	−	+	+
590. *S. inositophilus*	−	−	−	−	−	−	−	+	−	−	+	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	n	−	+	+	−	+	−	+	+
591. *S. kluyveri-nielii*	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	n	−	+	+	−	+	+	+	+
592. *S. lactophilus*	−	−	−	−	−	−	−	+	−	v	+	+	+	+	+	−	−	+	−	+	+	−	+	−	−	−	n	−	v	+	v	+	−	+	v
593. *S. oryzicola*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	+	−	−	+	+	+	+	−	−	n	−	−	+	−	+	−	+	+
594. *S. phyllomatis*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	−	−	−	+	−	−	+	+	+	−	+	−	n	−	+	+	−	+	+	+	+
595. *S. roseus*	−	−	−	−	−	−	−	+	v	v	v	+	v	v	−	−	+	v	−	v	v	v	v	v	v	−	n	−	v	v	−	v	v	+	v
596. *S. ruber*	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	w	+	+	−	−	−	−	−	−	−	−	n	−	−	w	−	+	−	+	+
597. *S. salicinus*	−	−	−	−	−	−	−	+	+	−	+	−	+	+	−	−	+	−	−	−	+	+	+	−	−	−	n	−	−	+	−	+	+	+	+
598. *S. sasicola*	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	−	+	−	−	+	+	+	−	−	−	−	n	−	−	−	−	w	−	+	w
599. *S. singularis*	−	−	−	−	−	−	−	+	−	−	−	−	+	+	+	−	−	−	−	−	−	v	+	−	−	−	n	−	+	+	−	+	−	+	+
600. *S. subbrunneus*	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−	−	+	−	−	−	−	−	+	−	−	−	n	−	−	+	−	+	−	+	+
601. *S. tsugae*	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	−	−	+	−	−	+	−	v	−	−	−	n	−	+	+	−	v	−	+	+
602. *S. xanthus*	−	−	−	−	−	−	−	+	w	−	+	+	+	+	w	−	+	−	−	+	−	+	−	−	−	−	n	−	−	−	−	+	−	+	+
Sporopachydermia																																			
603. *S. cereana*	−	−	−	−	−	−	−	+	−	+	−	−	v	v	−	−	−	−	−	−	+	v	−	v	−	−	−	−	+	+	+	+	−	+	+
604. *S. lactativora*	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	v	−	−	−	−	+	+	−	+	−	v	v
605. *S. quercuum*	w	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−
Stephanoascus																																			
606. *S. ciferrii*	−	−	−	−	−	−	−	+	+	+	+	+	v	+	−	v	+	−	−	v	+	+	+	v	+	−	n	−	v	+	+	+	+	+	+
607. *S. farinosus*	w	v	−	v	v	−	v	+	+	v	−	v	+	+	v	v	v	−	−	v	v	v	v	v	−	v	n	n	v	+	v	+	v	+	+
608. *S. smithiae*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	w	+	+	+	+	+	+	n	−	+	+	+	+	+	+	+
Sterigmatomyces																																			
609. *S. elviae*	−	−	−	−	−	−	−	+	−	+	+	−	+	+	+	−	+	−	−	−	+	+	+	+	−	−	v	−	+	+	+	+	+	+	+
610. *S. halophilus*	−	−	−	−	−	−	−	+	v	v	−	−	v	+	v	−	−	−	−	−	+	v	×	+	−	−	v	−	×	+	+	+	v	+	+
Sterigmatosporidium																																			
611. *S. polymorphum*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−	+	w	+	+	+	+	+
Sympodiomyces																																			
612. *S. parvus*	−	−	−	−	−	−	−	+	+	−	+	w	+	+	−	−	−	−	−	−	+	+	v	v	+	+	+	−	−	+	+	+	−	+	+
Sympodiomycopsis																																			
613. *S. paphiopedili*	−	−	−	−	−	−	−	+	w	+	+	+	+	+	w	+	+	+	w	w	+	+	+	+	−	n	n	n	n	+	+	−	−	+	+
Tilletiaria																																			
614. *T. anomala*	−	−	−	−	−	−	−	+	−	−	+	−	+	+	+	+	+	−	+	+	−	+	−	−	−	−	n	−	−	+	−	−	−	+	−
Tilletiopsis																																			
615. *T. albescens*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	−	n	−	v	+	+	+	−	+	+
616. *T. flava*	−	−	−	−	−	−	−	+	−	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	−	−	n	−	−	+	−	+	−	+	+
617. *T. fulvescens*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	n	−	v	+	+	+	−	+	+
618. *T. minor*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	n	−	v	+	v	+	−	+	+
619. *T. pallescens*	−	−	−	−	−	−	−	+	v	+	+	+	+	+	v	+	+	+	v	+	v	+	v	v	−	−	−	−	v	v	+	+	−	+	+
620. *T. washingtonensis*	−	−	−	−	−	−	−	+	v	−	+	+	v	+	−	v	+	+	−	+	+	+	+	+	−	−	n	−	v	+	+	v	−	+	+
Torulaspora																																			
621. *T. delbrueckii*	+	v	v	v	−	v	v	+	v	v	v	v	−	+	−	−	v	v	v	−	v	−	−	−	−	−	−	−	+	v	−	v	−	+	v
622. *T. globosa*	+	−	+	−	−	×	−	+	−	−	+	−	−	v	−	−	+	−	v	−	−	−	−	−	−	−	−	−	+	v	−	−	−	+	−
623. *T. pretoriensis*	+	×	+	+	−	v	v	+	+	−	+	+	−	+	−	−	+	v	+	−	−	−	−	−	−	−	−	−	×	v	−	−	−	+	v
Tremella																																			
624. *T. aurantia*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	n	−	+	+	−	+	v	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Sporobolomyces																																								
584.	−	+	+	+	+	+	−	n	−	−	−	+	n	n	+	+	+	+	−	v	−	v	v	v	n	−	+	n	v	−	+	+	+	+	n	10	63.0	+	n	+
585.	−	+	−	−	+	−	−	n	−	−	−	−	n	n	+	−	+	+	−	−	−	−	−	−	n	−	+	n	−	−	+	+	−	−	−	$10(H_2)$	56.5	+	n	−
586.	−	+	+	−	+	+	−	n	+	+	−	+	n	n	+	−	+	+	−	−	−	−	+	−	n	−	+	n	−	−	+	+	n	−	−	10	53.1	+	n	+
587.	−	−	+	−	w	−	−	n	+	+	−	+	n	n	+	+	w	+	−	−	−	−	−	−	n	−	+	n	−	−	+	+	n	−	−	10	56.0	+	n	−
588.	−	−	−	v	+	v	−	n	−	−	−	−	n	n	+	−	−	−	−	−	−	v	−	−	n	−	+	n	−	−	+	v	−	−	−	10	50.9	+	n	+
589.	−	+	+	v	+	+	−	n	+	+	−	+	n	n	+	−	+	+	−	−	−	−	+	−	n	−	+	n	+	−	+	+	−	−	−	10	60.9	+	n	−
590.	−	−	+	−	+	+	+	n	+	+	−	+	n	n	+	−	+	+	−	+	+	−	+	−	n	−	+	n	+	+	+	+	−	−	−	10	58.1	+	n	+
591.	−	−	+	−	+	+	−	n	+	+	−	+	n	n	+	+	−	−	−	−	−	+	−	−	n	−	+	n	+	−	+	+	−	−	−	10	53.8	+	n	+
592.	v	−	−	+	+	+	−	n	+	+	−	−	n	n	−	−	−	+	+	+	+	−	+	−	n	−	+	n	−	−	+	+	n	−	−	10	55.7	+	n	+
593.	+	w	+	+	+	+	−	n	−	−	−	+	n	n	+	−	+	+	−	−	−	−	−	−	n	−	+	n	−	−	+	+	n	−	−	10	61.1	+	n	−
594.	−	−	+	−	+	+	−	n	−	−	−	w	n	n	+	+	+	w	−	−	−	−	−	−	n	−	+	n	−	−	+	+	−	−	−	10	47.4	+	n	+
595.	v	v	v	v	+	v	−	n	+	+	v	−	n	n	v	v	v	v	−	v	−	v	v	−	n	−	+	n	v	v	+	+	n	−	−	10	52.2	+	+	v
596.	+	−	−	−	+	−	−	n	−	−	−	v	n	n	−	−	−	−	−	−	−	+	−	−	n	−	−	n	−	−	+	+	−	−	−	10	50.0	+	n	−
597.	−	w	−	−	+	+	−	n	+	+	−	−	n	n	+	+	+	+	−	w	−	v	−	−	n	−	+	n	−	−	+	+	n	−	−	10	51.1	+	n	+
598.	−	w	−	−	+	−	−	n	−	−	−	−	n	n	w	−	−	−	−	+	−	+	+	−	n	−	+	n	+	−	+	+	−	−	−	10	56.9	+	n	−
599.	−	+	+	+	+	+	−	n	−	−	−	+	n	n	+	−	−	+	−	+	−	−	+	−	n	−	+	n	w	−	+	+	−	−	−	10	58.0	+	n	−
600.	−	−	+	+	+	+	−	n	+	+	−	−	n	n	+	−	−	+	−	−	−	+	−	−	n	−	+	n	−	−	+	+	−	−	−	10	68.0	+	n	−
601.	v	+	+	+	+	+	−	n	+	+	−	+	n	n	v	−	+	+	+	−	−	+	+	−	n	−	+	n	−	−	+	+	n	−	−	10	50.9	+	n	−
602.	+	−	−	−	+	w	−	n	+	+	−	−	n	n	w	−	−	−	−	+	−	+	+	−	n	−	+	n	−	−	+	+	n	−	−	10	59.5	+	n	−
Sporopachydermia																																								
603.	−	+	−	v	v	−	+	−	−	−	−	−	n	n	+	n	n	n	n	+	n	+	+	−	−	−	n	−	+	v	+	+	+	+	+	9	49.6	−	−	−
604.	−	−	−	+	+	−	+	−	−	−	−	−	n	n	+	n	n	n	n	−	n	+	+	−	−	−	n	−	+	+	+	+	+	+	+	9	46.2	+	−	−
605.	−	−	−	w	v	−	+	−	−	−	−	−	n	n	+	n	n	n	n	+	n	+	+	−	−	−	n	−	+	+	+	+	+	−	−	9	37.8	+	−	−
Stephanoascus																																								
606.	v	v	+	+	v	v	+	n	−	v	−	+	+	n	+	+	v	n	n	+	−	+	+	+	n	−	−	n	+	+	+	+	+	+	n	9	46.6	−	n	+
607.	v	v	−	−	v	v	−	n	−	n	v	n	n	n	n	n	v	n	n	n	n	n	n	n	n	−	n	n	n	n	+	+	n	−	−	9	49.2	−	n	+
608.	+	+	+	−	+	+	+	+	+	+	+	+	n	−	+	n	+	n	+	+	−	n	+	−	+	−	−	n	+	+	+	+	+	−	−	9	47.0	−	n	+
Sterigmatomyces																																								
609.	−	+	+	v	+	+	−	−	−	n	−	+	+	n	n	n	+	n	n	n	n	n	v	+	n	−	+	n	−	−	+	+	+	+	n	9	52.0	+	−	−
610.	−	+	−	−	+	+	−	−	v	n	−	+	+	n	n	n	+	n	n	n	n	n	v	+	n	−	+	n	−	−	+	+	−	−	−	9	55.1	+	+	−
Sterigmatosporidium																																								
611.	+	+	+	−	+	+	+	−	−	n	−	+	+	+	n	n	n	n	n	n	n	n	−	−	+	+	+	−	n	n	+	+	n	−	n	10	51.9	+	−	v
Sympodiomyces																																								
612.	−	−	−	−	−	−	+	−	−	n	−	n	n	−	n	n	+	n	n	n	n	n	n	−	−	−	−	−	n	n	+	+	−	n	n	9	46.3	−	−	−
Sympodiomycopsis																																								
613.	+	−	n	w	+	−	+	n	+	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	n	−	w	n	n	n	n	+	n	−	n	10	56.3	+	n	v
Tilletiaria																																								
614.	−	−	−	−	+	−	−	n	−	+	+	−	n	n	n	n	n	n	n	n	n	n	n	−	n	−	+	n	n	n	+	+	−	−	−	10	62.9	+	−	+
Tilletiopsis																																								
615.	+	v	+	+	+	+	+	n	+	+	+	v	n	n	+	+	+	v	−	−	−	v	−	−	n	−	+	n	−	−	+	+	−	−	−	10	56.7	+	+	+
616.	−	−	−	+	+	+	−	n	+	+	−	−	n	n	+	+	+	−	−	−	−	+	+	−	n	−	+	n	−	−	+	+	n	−	−	10	63.2	+	+	+
617.	−	−	v	v	+	+	−	n	+	+	−	+	n	n	+	+	+	−	−	−	−	v	v	−	n	−	+	n	−	−	+	+	n	−	−	10	67.1	+	+	+
618.	v	−	v	+	+	+	−	n	+	v	−	v	n	n	+	+	+	v	v	−	−	v	v	−	n	−	+	n	−	−	+	+	n	−	−	10	56.0	+	−	+
619.	v	v	v	v	+	v	v	n	+	v	+	v	n	n	v	+	v	v	v	v	v	v	v	−	n	−	+	n	−	−	+	+	n	−	−	10	43.7	+	+	+
620.	v	v	+	+	+	+	−	n	+	+	−	−	n	n	+	+	v	v	−	−	−	v	−	−	n	−	+	n	−	−	+	+	n	−	−	10	68.2	+	+	+
Torulaspora																																								
621.	v	−	v	v	v	−	−	−	−	n	v	+	−	−	n	n	n	n	n	−	n	n	n	n	+	−	n	−	−	−	+	+	n	v	n	6	43.4	−	−	−
622.	−	−	v	+	−	−	−	−	−	n	+	+	−	−	n	n	n	n	n	−	n	n	n	n	v	−	n	−	×	−	+	+	+	+	n	6	47.3	−	−	−
623.	+	−	v	+	−	−	−	−	−	n	+	+	−	−	n	n	n	n	n	−	n	n	n	n	v	−	n	−	−	−	+	+	+	+	n	6	46.4	−	−	−
Tremella																																								
624.	+	+	+	+	+	v	+	n	−	−	−	+	n	n	n	n	n	n	n	n	n	n	n	v	n	+	+	n	n	n	+	+	−	−	−	n	n	+	n	v

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

Species	Fermentation							Assimilation reactions and other characteristics																												
	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Tremella (*cont'd*)																																			
625. *T. brasiliensis*	−	−	−	−	−	−	−	+	+	+	−	+	+	+	+	−	−	−	−	−	+	v	+	+	−	+	n	−	−	−	+	+	−	+	+
626. *T. cinnabarina*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+	+	+	−	+	n	−	+	+	+	+	+	+	+
627. *T. coalescens*	−	−	−	−	−	−	−	+	−	+	−	−	−	+	−	−	−	+	−	−	+	−	+	−	−	−	n	−	−	−	−	+	−	+	+
628. *T. encephala*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	v	v	+	−	+	+	+	+	+	+	+	n	−	+	+	−	+	+	+	+
629. *T. foliacea*	−	−	−	−	−	−	−	+	v	v	−	+	+	+	−	−	−	−	−	−	+	+	+	v	+	v	n	−	+	+	v	+	v	+	+
630. *T. fuciformis*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	n	−	+	+	+	+	+	+	+
631. *T. globispora*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+	−	+	+	+	+	+	−	+	n	−	+	+	+	+	+	+	+
632. *T. indecorata*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	v	v	v	+	−	v	+	+	+	+	+	+	n	−	+	v	−	+	+	+	+
633. *T. mesenterica*	−	−	−	−	−	−	−	+	−	+	−	−	+	+	+	−	−	−	−	−	+	−	+	−	−	−	n	−	+	−	+	+	−	+	+
634. *T. moriformis*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	−	+	+	−	−	+	+	+	+	+	+	n	−	−	+	−	+	+	+	+
Trichosporon																																			
635. *T. aquatile*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	−	−	v	−	+	+	+	v	+	−	−	+	−	+	−	+	−	−	−	−
636. *T. asahii*	−	−	−	−	−	−	−	+	+	v	v	+	+	v	+	−	−	v	−	v	v	v	+	+	+	+	+	−	+	v	+	v	−	v	v
637. *T. asteroides*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	v	+	−	+	+	+	v	−	v	v
638. *T. brassicae*	−	−	−	−	−	−	−	+	+	+	+	+	−	+	−	−	−	−	−	+	+	+	−	+	−	+	+	−	+	−	−	−	−	+	+
639. *T. coremiiforme*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	−	−	+	−	+	+	+	v	+	+	−	+	−	+	v	+	−	v	+	v
640. *T. cutaneum*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	+	+	+	−	+	+	+	v	+	+	v	+	−	+	+	+	+	−	+	+
641. *T. dulcitum*	−	−	−	−	−	−	−	+	−	v	+	+	+	+	+	−	+	+	−	v	+	+	v	+	+	v	+	−	v	+	−	−	v	+	+
642. *T. faecale*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	+	−	−	+	+	+	+	v	+	+
643. *T. gracile*	−	−	−	−	−	−	−	+	−	v	v	v	+	+	v	−	−	−	−	v	+	+	−	v	−	v	−	−	+	+	−	v	−	+	v
644. *T. inkin*	−	−	−	−	−	−	−	+	v	v	+	+	+	+	+	−	−	+	−	+	+	v	v	+	−	v	+	−	+	v	+	−	−	v	−
645. *T. jirovecii*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
646. *T. laibachii*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	v	−	+	+	+	v	+	+	+	+	−	+	+	−	v	+	v	v
647. *T. loubieri*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	+	+	−	−	+	+	+	v	+	+	+	+	−	+	+	−	+	−	+	+
648. *T. moniliiforme*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	+	+	+	−	+	+	+	v	+	+	v	+	−	+	+	+	+	−	+	+
649. *T. montevideense*	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	−	−	+	−	+	+	+	−	+	−	v	+	−	+	+	−	+	+	+	+
650. *T. mucoides*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
651. *T. ovoides*	−	−	−	−	−	−	−	+	+	v	+	+	+	v	+	−	v	v	−	+	+	v	v	+	+	v	+	−	+	v	+	−	−	+	v
652. *T. pullulans*	−	−	−	−	−	−	−	+	+	v	+	+	+	+	+	+	+	v	−	+	+	+	v	v	v	−	−	−	+	v	+	+	−	+	+
653. *T. sporotrichoides*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	−	+	+	+	+	−	+	+	−	−	−	+	+
Trichosporonoides																																			
654. *T. madida*	+	−	+	+	−	−	n	+	−	−	+	+	+	−	−	−	−	−	−	−	+	+	−	+	−	n	n	n	n	+	+	−	−	+	+
655. *T. megachiliensis*	+	−	+	+	−	−	n	+	−	−	+	+	+	−	−	−	−	−	−	v	−	−	−	v	−	n	n	n	n	+	+	−	−	+	v
656. *T. nigrescens*	+	+	−	+	−	−	n	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	n	n	n	+	+	+	−	−	+	+
657. *T. oedocephalis*	+	+	+	+	−	−	n	+	+	−	+	+	+	−	−	−	−	−	−	−	−	v	−	+	−	n	n	n	n	+	+	−	−	+	+
658. *T. spathulata*	+	+	+	+	v	−	n	+	v	−	+	+	+	−	v	−	+	+	−	−	v	−	−	+	−	n	n	n	n	+	+	−	−	+	+
Trigonopsis																																			
659. *T. variabilis*	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	−	−	−	v	−	−	+	−	−	n	−	+	+	−	−	−	+	+
Trimorphomyces																																			
660. *T. papilionaceus*	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	v	+	+	+	+	n	−	−	v	v	+	v	+	+
Tsuchiyaea																																			
661. *T. wingfieldii*	−	−	−	−	−	−	−	+	+	+	+	−	w	+	v	+	+	−	−	−	+	+	+	+	+	v	+	−	+	+	+	+	−	+	+
Ustilago																																			
662. *U. maydis*	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	n	−	+	+	+	+	+	+	−
Wickerhamia																																			
663. *W. fluorescens*	+	+	+	−	−	+	−	+	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	+	+	−	+	−	+	+
Wickerhamiella																																			
664. *W. domercqiae*	−	−	−	−	−	−	−	+	v	+	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	×	+	−	−	−	+	+
Williopsis																																			
665. *W. californica*	+	−	−	−	−	−	−	+	−	+	v	v	+	v	−	−	−	−	−	−	+	−	−	−	v	−	−	−	+	+	−	−	−	+	v
666. *W. mucosa*	+	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−	−	+	−	−	+	−	v	−	−	−	−	−	+	+	−	−	−	×	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
Tremella *(cont'd)*																																							
625.	−	+	−	−	+	v	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	−	−	−	n	n	+	n	v
626.	+	+	+	+	+	+	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	n	−	n	v	+	n	n	n	+	+	n	−	−	n	n	+	−	v
627.	−	−	−	−	+	+	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	−	−	−	n	n	+	n	v
628.	+	+	+	v	+	+	+	n	−	−	−	+	n	n	n	n	n	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	n	v	n	n	n	+	n	v
629.	−	−	+	v	+	+	+	n	−	+	−	+	n	n	n	n	n	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	n	−	−	n	n	+	n	v
630.	+	+	+	+	+	+	−	n	−	−	v	v	n	n	n	n	n	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	n	−	−	n	n	+	n	v
631.	+	+	+	+	+	+	−	n	−	−	−	+	n	n	n	n	n	n	n	n	n	n	n	−	n	v	+	n	n	n	+	+	−	−	−	n	n	+	n	v
632.	+	+	+	+	+	+	+	n	−	−	−	+	n	n	n	n	n	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	n	−	−	n	n	+	n	v
633.	−	+	+	−	+	+	−	n	−	−	−	−	n	n	n	n	n	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	n	−	−	n	n	+	n	v
634.	+	+	+	+	+	+	w	n	−	+	−	+	n	n	n	n	n	n	n	n	n	n	n	−	n	+	+	n	n	n	+	+	n	−	−	n	n	+	−	v
Trichosporon																																							
635.	+	+	+	+	+	+	−	n	−	−	−	+	+	n	−	−	+	n	n	+	−	+	+	+	+	n	+	n	+	+	n	+	−	−	−	9	62.6	+	n	+
636.	+	+	+	v	+	+	v	n	−	v	−	+	+	n	v	+	+	n	n	+	−	+	+	+	w	n	+	n	+	v	n	+	+	+	+	9	58.2	+	n	+
637.	+	v	+	+	+	+	+	n	−	−	−	+	+	n	+	+	v	n	n	+	−	+	+	+	w	n	+	n	v	v	n	+	n	v	−	9	61.7	+	n	+
638.	−	−	+	+	+	+	+	n	−	−	−	+	+	n	−	−	−	n	n	+	−	+	+	+	w	n	+	n	+	−	n	+	+	−	−	9	54.4	+	n	+
639.	+	+	+	+	+	v	+	n	−	+	−	+	+	n	+	+	+	n	n	+	−	+	+	+	w	n	+	n	+	+	n	+	+	+	n	9	59.3	+	n	+
640.	+	+	+	+	+	+	+	n	−	−	−	+	+	n	+	+	+	n	n	+	−	+	+	−	−	n	+	n	v	−	n	+	n	−	−	10	60.8	+	n	+
641.	−	v	+	+	+	v	+	n	−	−	−	+	+	n	+	−	+	n	n	+	−	+	+	−	−	n	+	n	+	−	n	+	−	−	−	9	55.2	+	n	+
642.	+	+	+	+	+	+	+	n	−	−	−	+	+	n	+	+	+	n	n	+	−	+	+	+	−	n	+	n	+	+	n	+	+	+	n	10	58.8	+	n	+
643.	−	+	+	+	+	+	+	n	−	−	−	+	+	n	v	−	v	n	n	+	−	+	+	v	−	n	+	n	+	+	n	+	n	−	−	9	57.7	+	n	+
644.	+	v	+	+	+	+	+	n	−	v	−	+	+	n	−	−	+	n	n	+	−	+	+	w	−	n	+	n	+	v	n	+	+	+	+	9	59.3	+	n	+
645.	+	+	+	+	+	+	+	n	−	−	−	+	+	n	+	+	+	n	n	+	−	+	+	+	−	n	+	n	+	v	n	+	n	−	−	10	61.4	+	n	+
646.	+	+	+	+	+	+	+	n	−	v	−	+	+	n	v	v	+	n	n	+	+	+	+	−	−	n	+	n	+	+	n	+	n	n	n	9	57.3	+	n	+
647.	+	+	+	+	+	+	+	n	−	−	−	+	+	n	v	+	+	n	n	+	−	+	+	+	−	n	+	n	+	+	n	+	+	+	+	9	56.7	+	n	+
648.	+	+	+	+	+	+	+	n	−	v	−	+	+	n	+	+	+	n	n	+	−	+	+	−	−	n	+	n	+	−	n	+	n	−	−	10	58.5	+	n	+
649.	+	+	+	+	+	+	+	n	−	−	−	+	+	n	+	v	+	n	n	+	−	+	+	+	−	n	+	n	+	−	n	+	+	−	−	9	55.2	+	n	+
650.	+	+	+	+	+	+	+	n	−	v	−	+	+	n	+	+	+	n	n	+	−	+	+	w	w	n	+	n	+	v	n	+	+	+	n	10	58.6	+	n	+
651.	+	+	+	+	+	v	+	n	−	v	−	+	+	n	v	−	+	n	n	+	−	+	+	w	−	n	+	n	+	−	n	n	n	v	n	9	58.4	+	n	+
652.	+	v	+	−	+	+	+	n	+	+	v	+	+	n	+	−	+	n	n	v	−	v	v	v	w	n	+	n	−	−	n	v	−	−	−	9	57.0	+	n	+
653.	+	+	+	+	+	+	+	n	−	+	−	+	+	n	−	+	+	n	n	+	+	+	+	−	−	n	+	n	−	−	n	+	−	−	−	9	61.5	+	n	+
Trichosporonoides																																							
654.	−	−	n	−	+	+	−	n	+	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	+	+	+	n	9	50.2	+	n	v
655.	−	−	n	−	v	+	−	n	v	n	n	n	n	n	n	n	n	n	n	+	n	+	v	+	n	n	n	n	n	n	+	+	+	+	n	9	n	+	n	+
656.	−	−	n	−	−	−	−	n	+	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	+	n	−	−	9	57.0	+	n	v
657.	−	−	n	−	v	v	−	n	+	n	−	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	+	+	+	n	9	45.8	+	n	+
658.	−	+	n	+	+	+	−	n	+	n	+	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	+	+	+	n	9	62.0	+	n	+
Trigonopsis																																							
659.	−	−	−	+	+	+	−	n	−	−	−	−	n	n	+	−	−	−	−	+	−	v	+	−	n	−	−	n	+	+	+	+	+	+	−	n	46.1	−	−	−
Trimorphomyces																																							
660.	+	+	+	+	+	+	v	n	−	−	−	+	n	n	n	n	n	n	n	n	n	n	n	−	n	v	+	n	n	n	+	+	−	−	−	n	n	+	−	v
Tsuchiyaea																																							
661.	−	+	+	−	v	−	+	−	−	n	−	+	−	n	n	n	+	n	n	n	n	w	−	+	n	w	+	n	−	−	+	+	−	−	−	9	54.4	+	+	−
Ustilago																																							
662.	+	+	+	+	+	−	+	n	+	+	+	+	n	−	n	n	n	n	n	n	n	n	n	−	n	−	+	n	n	n	+	+	+	+	n	10	n	+	−	+
Wickerhamia																																							
663.	−	−	w	w	+	+	−	+	−	−	−	+	−	n	n	n	n	n	n	+	n	+	+	+	+	−	−	−	+	n	+	+	n	−	−	9	37.6	−	−	−
Wickerhamiella																																							
664.	−	−	+	−	+	w	−	−	+	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	+	−	n	−	n	n	+	+	n	−	−	n	48.6	−	−	−
Williopsis																																							
665.	v	×	+	+	+	v	−	−	+	n	−	v	v	−	n	n	n	n	n	n	n	n	n	n	−	−	n	v	n	n	n	+	n	−	n	7	43.0	−	v	−
666.	+	×	−	−	v	−	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n	n	n	n	v	−	n	−	n	n	n	+	n	−	n	7	34.0	−	+	−

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

	Fermentation							Assimilation reactions and other characteristics																											
Species	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	D-Glucosamine	*N*-Acetyl-D-glucosamine	Methanol	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol
Williopsis (*cont'd*)																																			
667. *W. pratensis*	+	−	w	−	−	−	−	+	+	−	+	+	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	+	+
668. *W. salicorniae*	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	+	−	+	+	+	+	−	+	+
W. saturnus																																			
669. var. *saturnus*	+	−	+	−	−	×	−	+	−	−	+	v	+	v	−	−	+	v	v	−	+	−	−	−	v	−	−	−	+	+	−	−	−	v	v
670. var. *mrakii*	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	v	−	−	−	+	+	−	−	−	v	v
671. var. *sargentensis*	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+	+	−	−	−	+	+
672. var. *suaveolens*	+	−	+	−	−	×	−	+	−	−	+	−	+	−	−	−	+	−	+	−	+	−	−	−	−	−	−	−	+	+	−	−	−	v	+
673. var. *subsufficiens*	+	−	+	−	−	+	−	+	−	−	+	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	+	+	−	−	−	+	+
Xanthophyllomyces																																			
674. *X. dendrorhous*	+	−	×	×	−	×	×	+	−	v	+	+	+	+	−	−	+	+	−	×	+	+	v	v	−	−	−	−	v	v	−	v	−	+	v
Yarrowia																																			
675. *Y. lipolytica*	−	−	−	−	−	−	−	+	v	v	−	−	v	−	−	−	−	−	−	−	−	−	−	v	−	−	+	−	+	+	+	v	−	+	+
Zygoascus																																			
676. *Z. hellenicus*	+	v	+	v	−	v	+	+	+	+	+	+	+	+	v	−	+	v	−	v	+	+	v	v	+	+	n	−	v	+	−	+	v	+	+
Zygosaccharomyces																																			
677. *Z. bailii*	+	−	v	−	−	−	v	+	v	v	v	−	−	×	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	×	−	v	−	+	+
678. *Z. bisporus*	+	−	−	−	−	−	−	+	v	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	v	−	+	+
679. *Z. cidri*	+	+	+	+	−	+	+	+	+	+	+	+	−	+	−	+	+	−	+	−	v	−	−	−	−	−	−	−	+	+	−	−	−	+	+
680. *Z. fermentati*	+	+	+	+	−	v	+	+	+	+	+	+	v	+	−	v	+	+	v	−	v	−	−	−	−	−	−	−	+	v	−	−	−	+	+
681. *Z. florentinus*	+	+	+	+	−	+	+	+	+	+	+	+	−	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	+	v	−	−	−	+	+
682. *Z. mellis*	+	−	v	×	−	−	−	+	−	−	−	v	−	v	−	−	−	−	−	−	v	−	−	−	−	−	−	−	−	+	−	−	−	+	+
683. *Z. microellipsoides*	+	+	+	−	−	+	−	+	+	v	+	−	−	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−	×	v	−	−	−	+	v
684. *Z. mrakii*	+	+	+	−	−	+	−	+	+	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	v	+	−	−	−	+	+
685. *Z. rouxii*	+	−	v	+	−	−	v	+	v	−	v	v	−	×	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	×	−	v	−	×	+
Zygozyma																																			
686. *Z. arxii*	−	−	−	−	−	−	−	+	+	+	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	n	−	−	+	−	−	−	−	−
687. *Z. oligophaga*	−	−	−	−	−	−	−	+	+	v	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	n	−	+	+	−	−	+	−	+
688. *Z. smithiae*	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	n	−	+	+	−	−	−	−	−
689. *Z. suomiensis*	−	−	−	−	−	−	−	+	+	+	−	−	+	−	+	−	−	−	−	−	+	+	+	+	−	−	n	−	+	+	−	+	−	+	+

Symbols: +, positive; –, negative; w, weak; ×, positive or weak; v, variable (+/–, w/–); n, no data

Assimilation reactions and other characteristics

	α-Methyl-D-glucoside	Salicin	D-Gluconate	DL-Lactate	Succinate	Citrate	Inositol	Hexadecane	Nitrate	Nitrite	Vitamin-free	2-Keto-D-gluconate	5-Keto-D-gluconate	Saccharate	Xylitol	L-Arabinitol	Arbutin	Propane 1,2 diol	Butane 2,3 diol	Cadaverine	Creatinine
***Williopsis* (*cont'd*)**																					
667.	+	+	+	+	+	+	−	−	+	n	−	+	−	−	n	n	n	n	n	n	n
668.	−	−	−	−	−	−	−	+	−	n	−	−	−	−	n	n	n	n	n	n	n
669.	v	v	+	+	+	v	−	−	+	n	+	−	−	−	n	n	n	n	n	n	n
670.	−	+	+	+	+	−	−	−	+	n	+	−	−	−	n	n	n	n	n	n	n
671.	−	+	−	+	+	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n
672.	−	+	+	+	+	−	−	−	+	n	+	−	−	−	n	n	n	n	n	n	n
673.	−	+	×	+	+	×	−	−	+	n	−	−	−	−	n	n	n	n	n	n	n
Xanthophyllomyces																					
674.	v	+	+	v	+	v	−	−	−	n	−	+	w	n	w	n	+	n	n	w	−
Yarrowia																					
675.	−	v	v	+	+	+	−	+	−	n	−	−	−	−	n	n	n	n	n	n	n
Zygoascus																					
676.	v	+	+	v	v	v	+	n	−	−	−	−	+	n	+	v	+	−	−	+	−
Zygosaccharomyces																					
677.	−	−	−	−	−	−	−	−	−	n	−	v	−	−	n	n	n	n	n	n	n
678.	−	−	v	−	−	−	−	−	−	n	−	v	−	−	n	n	n	n	n	n	n
679.	+	−	−	+	+	−	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n
680.	+	v	v	+	+	−	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n
681.	+	−	v	−	+	−	−	−	−	n	v	+	−	−	n	n	n	n	n	n	n
682.	−	−	v	−	−	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n
683.	−	−	+	+	v	−	−	−	−	n	−	+	−	−	n	n	n	n	n	n	n
684.	−	−	−	−	+	−	−	−	−	n	+	+	−	−	n	n	n	n	n	n	n
685.	−	−	v	−	−	−	−	−	−	n	−	−	−	−	n	n	n	n	n	n	n
Zygozyma																					
686.	−	+	−	−	+	−	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n
687.	−	−	+	−	+	−	v	n	−	n	−	n	n	n	n	n	n	n	n	n	n
688.	−	+	−	−	+	−	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n
689.	−	+	+	−	−	+	−	n	−	n	−	n	n	n	n	n	n	n	n	n	n

	L-Lysine	Ethylamine	50% Glucose	10% NaCl/5% glucose	Starch formation	Urease	Gelatin liquefaction	0.01% Cycloheximide	0.1% Cycloheximide	Growth at 19°C	Growth at 25°C	Growth at 34°C	Growth at 37°C	Growth at 40°C	Co-Q (Main component)	Mol% G+C (Ave.)	DBB	Pellicle	True Hyphae
***Williopsis* (*cont'd*)**																			
667.	n	n	n	−	−	n	−	n	n	n	+	n	−	n	n	39.9	−	w	−
668.	n	n	n	−	−	n	−	n	n	n	+	n	−	n	n	36.7	−	−	−
669.	n	n	n	−	−	n	v	n	n	n	+	n	v	n	7	44.4	−	+	−
670.	n	n	n	−	−	n	v	n	n	n	+	n	v	n	7	44.3	−	+	−
671.	n	n	n	−	−	n	−	n	n	n	+	n	−	n	n	44.1	−	+	−
672.	n	n	n	−	−	n	−	n	n	n	+	n	−	n	n	44.6	−	+	−
673.	n	n	n	−	−	n	v	n	n	n	+	n	−	n	7	43.6	−	+	−
Xanthophyllomyces																			
674.	×	−	w	−	+	+	w	−	−	+	v	−	−	−	10	48.3	+	+	−
Yarrowia																			
675.	n	n	n	−	−	+	+	n	n	+	+	+	v	n	9	49.9	−	+	+
Zygoascus																			
676.	+	+	+	n	−	−	n	+	+	+	+	+	+	n	9	43.9	−	v	+
Zygosaccharomyces																			
677.	n	n	n	+	−	n	−	n	−	+	+	n	v	n	6	44.4	−	−	−
678.	n	n	n	+	−	n	−	n	−	+	+	n	−	−	6	45.3	−	−	−
679.	n	n	n	+	−	n	−	+	+	+	+	n	−	−	6	43.6	−	−	−
680.	n	n	n	+	−	n	−	+	+	+	+	+	+	n	6	44.2	−	−	−
681.	n	n	n	−	−	n	−	+	+	+	+	n	−	−	6	43.0	−	−	−
682.	n	n	n	+	−	n	−	n	−	+	+	n	−	−	6	41.4	−	−	−
683.	n	n	n	−	−	n	−	n	−	+	+	n	−	−	6	41.4	−	−	−
684.	n	n	n	+	−	n	−	+	+	+	+	n	−	−	6	42.2	−	−	−
685.	n	n	n	+	−	n	−	n	−	+	+	n	v	n	6	41.7	−	−	−
Zygozyma																			
686.	n	n	n	n	+	n	n	+	+	+	+	−	−	−	9	47.4	−	−	−
687.	n	n	n	n	+	n	n	+	+	+	+	−	−	−	8	41.7	−	−	−
688.	n	n	n	n	+	n	n	+	+	+	+	−	−	−	9	55.5	−	−	−
689.	n	n	n	n	+	n	n	+	+	+	+	−	−	−	8	45.1	−	−	−

Symbols: +, positive; −, negative; w, weak; ×, positive or weak; v, variable (+/−, w/−); n, no data

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Glossary of terms used in this book

abstricted: formation of spores by the cutting off of successive portions of the sporophore through growth of the septa

acerose: needle-shaped

acicular: needle-shaped, see acerose

acropetal: produced successively in the direction of the apex; the apical part is youngest

aerobic: free oxygen is required for growth; an atmosphere with free oxygen

agglutinate: clump or bind together

allantoid: curved with rounded ends, sausage-shaped

allele: any of the alternative expressions of a gene

allotype: paratype of the opposite sex to the holotype

amphigenous hymenium: spore layer of fruit body formed on two sides

amphidiploidy (allopolyploidy): production of a fertile species by the fusion of the genomes of two separate but usually related species with different chromosome numbers; the new species is reproductively isolated from the parent species

ampulliform: flask-like form; shaped like a Florence flask

anamorph (anamorphic state): asexual part of life cycle, mitotic state

anastomosis: fusion between hyphae

annellation: formation of ring-like protrusions near the apex of a cell resulting from successive formation of conidia at the same site

annellidic: having annellations

apex: end of a cell farthest from base or point of attachment

apical: referring to apex

apiculate: cell with a short projection (**apiculum**) at one or both ends of a cell

arthroconidium (arthrospore): an asexual spore resulting from the division of a hypha or a single cell by fission, for example, *Trichosporon*

ascophore: ascus-bearing hypha

ascus: the sac-like cell in which ascospores are produced

autogamy: fusion of nuclei within a cell in the absence of cell fusion

axenic culture: a pure culture of a single organism

ballistoconidium: an asexual ballistospore

ballistospore: spore that is forcibly projected from a basidium

base composition of DNA: molar ratio of guanine + cytosine (G+C) or adenine + thymine (A+T) expressed as the percent that the particular base pair comprises of a DNA molecule

basidiocarp: the teleomorphic reproductive structure of a basidiomycete

basidiophore: basidium-bearing hypha

basidiospore: a spore produced by a basidium

basidium: organ of the teleomorphic (sexual) state of a basidiomycete that produces basidiospores

basipetal: produced successively in the direction of the base; the apical part is oldest

biallelic incompatibility: two alleles per mating locus, for example, A_1 and A_2 or a and α, ascomycetes and basidiomycetes

bifactorial mating system: see tetrapolar incompatibility system

bilaterally symmetrical: with one plane of symmetry

bipolar incompatibility (unifactorial): a system with a single incompatibility locus; bipolar, because of the two incompatibility groups found among the progeny of a cross; may be biallelic or multiallelic

bisexual: two opposite mating types in the genome of a single cell, a heterothallic diploid

blastese: non-septate cells that resemble true mycelium, found in the genera *Brettanomyces* and *Dekkera*

blastoconidium: a vegetative spore formed by enlargement of the bud initial that precedes septal formation

blastospore: see blastoconidium

botuliform: cylindrical with rounded ends

bud: formation of a new cell from an outgrowth on the parent cell on a broad or narrow base

budding:

- *monopolar*: forming buds at only one pole of the cell, usually on a broad base
- *bipolar*: forming buds at both poles of the cell, usually on a broad base
- *polar*: forming buds at one or both poles of the cell, usually on a narrow base or on a denticle
- *multipolar (multilateral)*: forming buds, usually on a narrow base, over nearly the entire surface of the cell

bud-fission: bud formation on a broad base with cross-wall development at the constriction between bud and parent cell; typical yeasts with bipolar budding are *Nadsonia, Saccharomycodes, Hanseniaspora* (*Kloeckera*) and *Wickerhamia*

bullate: spores with a rounded projection

capitate: with a spherical tip

capsule: a hyaline sheath of polysaccharide surrounding the yeast cell

catenulate: in a chain

cerebriform: brain-like appearance

chlamydospore: a thick-walled, intercalary or terminal asexual spore
clamp connection: a hyphal outgrowth formed during cell division of dikaryotic hyphae in basidiomycetes that makes a connection between the two cells by anastomosis. The function is to separate dividing nuclei during cell division; one of the daughter nuclei passes through the clamp.
clavate: club-shaped, broadest at the apex
clone: the asexual offspring from a single cell
closure line: the site in the center of the septum where, during growth, the septum has closed; there is a double layer of plasmalemma visible in a longitudinal section through the center of the hypha
coenzyme Q number: the number of isoprene units in the side chain of coenzyme Q (ubiquinone)
collarette: cup-shaped structure found at the apex of certain conidiogenous cells such as on the conidiophore of *Phialophora* or after successive budding at the same site on the cells of certain yeasts
compatible mating types: see complementary mating types
complementary mating types: strains (mating types) capable of mating to initiate a fertile dikaryotic or diploid state
congeneric: belonging to the same genus
conidiophore: a specialized hypha on which conidia are produced
conidium (conidiospore): an anamorphic spore
conjugated ascus: ascus developed from the fusion of two cells
conjugation: fusion of two cells during the mating process
conspecific: belonging to the same species
coralloid: made up of erect, branched or unbranched structures, coral-like
coremium: a fascicle of conidiophores; synnema
cornute: horned, horn-shaped
crescentiform: see lunate
crescent-shaped: see lunate
crispulate: with a wavy margin
cross: to bring together genetic material from different individuals
cross wall: see septum
cupulate: cup-shaped
cystidium: a sterile cell situated among the basidia and usually projecting beyond them in the hymenium in basidiomycetes

dacryoid: teardrop, tear-shaped; one end rounded and the other more or less pointed
dactyloid: finger-like
dangeardien: a structure (asci or basidia) in which karyogamy and meiosis take place
degree Balling: the gravity of wort that indicates sugar content by weight. The measurement is made with a saccharometer graduated with a scale that was constructed in 1843 by Carl Joseph Napoleon Balling. Degree Balling is similar to degree Brix, except that Brix was determined at 15°C whereas Balling was determined at 17.5°C. A saccharometer is a hydrometer consisting of a long graduated stem attached to an elongated bulb, which is weighted at the bottom. The hydrometer is placed in the wort and the gravity can be read on the graduated stem; the lower the content of sugar the deeper the meter sinks into the liquid.
dehiscent, dehiscing: opening when mature by pores or by becoming broken in parts
deliquescent ascus: see evanescent ascus
dentate: with tooth-like projections
denticle: a small tooth-like projection
diclavate: club-shaped at both ends
dikaryon: cells containing two genetically distinct nuclei
dikaryotic: see dikaryon
dimorphic: having two forms, e.g., budding cells and hyphae
diobovate: ovate at both ends with a central narrowing
diplo-haplontic life cycle: the cells reproducing vegetatively may be haploid or diploid, e.g., *Saccharomyces cerevisiae*
diploid: nucleus with a double set (2n) of chromosomes
diplontic life cycle: the vegetative cells are diploid, e.g., *Saccharomycodes ludwigii*
diplophase: the part of the life cycle in which the cells are diploid
distal: terminal, away from the point of origin
DNA: deoxyribonucleic acid
DNA reassociation: the pairing or reannealing of complementary single strands of DNA to form a double-stranded molecule (duplex)
DNA sequence homology: the extent of homology or relatedness between the sequences of nucleotides on complementary DNA molecules
DNA sequence relatedness: see DNA sequence homology
dolipore/parenthesome septum: a septal pore complex in the basidiomycetes in which the pore margin is enlarged into a barrel-like morphology (dolipore) and the paraseptal cytoplasm on either side is delimited by endoplasmic-reticulum-derived membrane domes (parenthesomes)
dormant: in state of rest

echinulate: with more or less pointed projections
effuse: growing flat
ellipsoidal: elliptical in optical section
ellipsoidopedunculate: stalk-like and ellipsoidal
endospore: an anamorphic spore formed within a vegetative cell or with a basidium
enteroblastic: involvement of the inner part of the cell wall of the parent cell in the formation of the bud
epibasidium: a structure located between a probasidium and basidiospores

epithet: the second (specific) part of a Latin binomial
erose: having delicate tooth-like projections from the edge
evanescent ascus: an ascus that breaks down when mature and liberates the ascospores
exosporium: the outer layer of the spore wall

falcate: sickle-shaped
farinose: with a powdery appearance
fascicle: bundle
fasciculate: growth in fascicles
filament (filamentous): thread-like hyphal or pseudohyphal cell
fimbriate: with a torn or minutely fringed margin
foliaceous: with a leaf-like appearance
fusiform: spindle-like
fusoid: spindle-like

G + C content: mol percent guanine + cytosine (G+C) of DNA; see also base composition of DNA
galeate: hat-shaped
gametangiogamy: fusion of gametangia
gametangium: gamete producing cell; alternatively, when gametes are not formed, copulation or conjugation of gametangia occurs
gelatinous: jelly-like
genotype: the genetic constitution of an individual taxon or cell
glabrous: smooth, not hairy or roughened

haploid: having a single set (n) of chromosomes
haplontic life cycle: the vegetative cells are haploid
haustorium : a special hyphal branch, some portion of which penetrates a host cell and absorbs nutrients
heterogamous conjugation: conjugation of two cells of different form or size
heterothallic: sexual reproduction requires the interaction of different mating types
hirsute: having long hairs
holoblastic: involvement of the entire cell wall of the parent cell in the formation of the bud
holometabasidium: non-septate metabasidium
holomorph: all stages of a fungus comprising the teleomorph and anamorph
holotype: material or strain indicated as nomenclatural type by the original author
homokaryon: a strain in which all nuclei are of the same genotype
homokaryotic fruiting: formation of a basidiomycete "teleomorph" in the absence of a dikaryophase; the mycelial cells are uninucleate and lack clamp connections
homonym: the same name given to two or more different taxa of the same rank based on different nomenclatural types
homothallic: sexual reproduction without the preceding interaction of complementary mating types

hyaline: transparent or colorless
hybrid: progeny of a cross involving genetically different parents
hymenium: spore bearing layer of an ascomycete or basidiomycete fruit body
hypha: one of the filaments of a mycelium

ICBM: International Code of Botanical Nomenclature
imperfect state: see anamorph
incompatibility: inability of two sexual strains to sexually reproduce
intercalary cell: a cell between two other cells
interfertility: mating of two organisms with production of fertile offspring
intramatrical : living in the matrix or substratum
isogamous: conjugating cells of the same shape and size
isotype: a duplicate of the holotype from the same, single collection as the holotype

karyogamy: the fusion of the two sex nuclei after plasmogamy

lateral: at the side
lectotype: a specimen designated as the nomenclatural type when no holotype was indicated at the time of publication, if the holotype is missing, or if the holotype is found to belong to more than one taxon
lenticular: shape of a double convex lens
life cycle: series of stages or events between the zygote of one generation and the subsequent zygote of the next generation
lobate: lobed
lobiform: in the shape of a lobe
locus: the site of a gene on a chromosome
lunate: like a crescent moon
lyophilization: a method of preserving viable, metabolically inactive cultures by freeze drying

mating: fusion of two cells that are sexually reactive
mating types: cells of opposite types, which are able to conjugate
meiosis: reduction division associated with sexual reproduction
meiosis bud: a bud attached to the parent cell which serves as the site of meiosis after fusion of the bud and parent cell nuclei
mesophilic: optimum temperature of growth is 20-40°C
metabasidium: the part of the basidium in which meiosis occurs
micropore: narrow pore-like structure in a septum
mitosis: nuclear division with retention of the original number of chromosomes in each daughter nucleus
multiallelic incompatibility: more than two alleles per incompatibility locus; more than two alleles for control of a particular function, for example, A_1, A_2, A_3 and A_1 B_1, A_2 B_3, basidiomycetes

mycelium: a mass of hyphae of an individual fungus; see also true- and pseudomycelium

neotype: strain or material designated as type when the material or strain on which the original description was based no longer exists

new combination, combinatio novo, comb. nov.: name of a taxon, which has been transferred in rank or position such as from one genus to another; term used by authors when new combination is made

nomen conservandum, nom. cons.: conservation of the name of a genus or species, although technically not acceptable, which has been approved by an International Botanical Congress

nomen invalidum, nom. inval. invalid name: a name, which has not been validly published, i.e., in accordance with the ICBN

nomen nudum, nom. nud.: a name published without an adequate description or diagnosis, i.e., in accordance with the ICBN

nuclear segregation: the segregation of genetically different nuclei contained in a heterokaryotic multikaryon

numerical analysis, numerical taxonomy: taxonomic arrangement based upon quantitation of phenotypic similarities and differences

obclavate: club-shaped with the broadest part at the base

oblate: flattened at the poles

oblong: twice as long as wide and having somewhat truncate ends

obovate: inversely ovate, narrowest at the base

obovoid: see obovate

obpyriform: pear-shaped but attached at the broad end

obtuse: rounded or blunt

ogival: pointed like a Gothic arch

orbicular: spherical

osmophilic: able to grow in a medium with elevated, i.e., greater than usual, sugar- or salt concentration

osmotic medium: medium with elevated sugar or salt concentration

osmotolerance: resistance to osmotic pressure

ovoid: egg-like, i.e., with narrowest end near tip

paraphysis: sterile filaments in the hymenium of ascomycetes and basidiomycetes; in the latter they are dikaryotic and allied to basidia, cystidia or hyphae

parasexuality: recombination of hereditary properties based not on sexual reproduction (meiosis) but on mitotic non-disjunction

paratype: strain other than the holotype on which the original description of a species is based

parenthesome: in basidiomycetes a curved, double membrane-derived structure differentiated from the endoplasmic reticulum that occurs on either side of the septal dolipore

pedicel: a small stalk-like structure

peduncle: stalk

perfect state: see teleomorph

persistent ascus: ascus not deliquescing (dissolving) at maturity

phenotype: the totality of characteristics of an individual as a result of the interaction between expressed genotype and environment

phragmometabasidium: a metabasidium divided by septa

pileus: the cap or hymenium-supporting part of the complex basidiomata or ascomata, e.g., agarics or morels

plasmodesmata: isthmus-like strands of protoplasm connecting adjacent cells; in hyphal septa also designated as micropores

plasmogamy: fusion of the cytoplasm of two compatible cells

plicate: folded into pleats

probasidium: the morphological part or developmental stage of the basidium in which karyogamy occurs

promycelium: see metabasidium

protosterigma: basal, filamentous or inflated part of the sterigma

prototrophic: growth factor-independent

pseudohyphae: see pseudomycelium

pseudomycelium: a linear arrangement of budding cells that remain attached to each other

psychrophilic: optimum temperature for growth is below 20°C

pulcherrima cells: round cells containing a large oil drop as with *Metschnikowia (Candida) pulcherrima*

pulcherrimin: a red iron-containing compound produced by some strains of *Metschnikowia, Kluyveromyces*, and certain other yeasts

pulvinate: cushion-like

pyriform: pear-shaped

reannealing experiments: see DNA reassociation

reniform: kidney-like shape

reticulate: net-like

rotational symmetry: with all planes of symmetry through the length axis

rugose: wrinkled

saccate: like a sack or bag

Saturn-shaped: spherical with a ledge around the middle, i.e., like the planet Saturn

self-fertile: see homothallic

septum: a cross wall in a hypha

sessile: lacking a stem or stalk

seta: a stiff hair or bristle

sib mating: mating or crossing of siblings

simple pore: central pore with the septum tapering

somatogamous conjugation: fusion of two whole cells

sphaeropedunculate (capitate): spheroidal end on an elongated cell

sporidium: see basidiospore
sporophore: a spore-producing or supporting structure
stalagmoid: like a long tear or drop
stemonitoid: with capitate synnema
sterigma: an extension (process) of a cell that supports a basidiospore
sterigmate: originating on sterigma
stilbelloid: with coremia
stipitate capitate: see sphaeropedunculate, but stipe may be long
sympodial proliferation: see sympodium
sympodium: a conidiophore that extends in growth by a succession of apices; a spore is produced at each apex and the growth continues to the side of the apex; the result is a zigzag appearance
syngamy: fusion of sexual cells resulting in karyogamy and zygote formation
synnema: see coremium

teleomorph, teleomorphic state: the state of the life cycle in which asco- or basidiospores are formed after nuclear fusion; meiotic state
teliospore: a thick-walled spore, which is a probasidium
tetrapolar incompatibility system (bifactorial mating system): a system with two incompatibility loci, *A* and *B*, which control mating competence; tetrapolar, because of the four incompatibility groups found among the progeny of a cross, usually multiallelic basidiomycetes
tremelloid: gelatinous, jelly-like
tremelloid basidium: cruciately septate phragmobasidium, i.e., having longitudinal and/or oblique septa
true hyphae: see true mycelium
true mycelium: a body of hyphal cells with apical growth and usually, cross walls
truncate: ending abruptly as though the end was cut off
tubercle: small wart-like structure; tuberculate with tubercles
turbinate: top-like in form
type material: specimen material on which the name of a taxonomic group, i.e., a species is based; may be dried herbarium material or a viable, metabolically inactive culture preserved by lyophilization or liquid nitrogen freezing
type species: the species which was designated as the nomenclatural type of a genus
type strain: the preserved, original specimen designated as the nomenclatural type

unifactorial mating system: see bipolar incompatibility system
unisexual: vegetative cells of a single mating type

velvety: having a velutinous appearance, i.e., densely covered with fine hairs
ventricose: swelling out in the middle or at one side; inflated
verrucose: having small rounded processes or warts
verticil: whorl
vesicle: a bladder-like sac, cell or cavity; a thin-walled body
vesiculate: with many vesicles

wale-like: ascospore ornamentation comprised of short ridges, which may be irregularly placed
Woronin bodies: membrane bound refractive spherical bodies occurring on either side of the pore of an ascomycete septum

zygote: a diploid cell that is the result of the fusion of two sexual cells or nuclei

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Index of taxa by genus and species

The names of genera, species and varieties accepted by the authors are indicated in bold type. The numbers of the pages referring to diagnoses and standard descriptions are printed in bold type as well.

Index to species and varietal names

Recurrent use of species and variety names, as well as new combinations from taxonomic changes, result in placement of the same name with different genera. Combinations accepted by authors of the present edition are given in bold type. Page numbers to each combination are given following the genus name.

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